ELSEVIER

Aquaculture 208 (2002) 191–364

Aquaculture

www.elsevier.com/locate/aqua-online

Review article

Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences

Robert H. Devlin [a,*], Yoshitaka Nagahama [b]

[a] *West Vancouver Laboratory, Fisheries and Oceans Canada, 4160 Marine Drive, West Vancouver, B.C., Canada V7V 1N6*

[b] *Laboratory of Reproductive Biology, Department of Developmental Biology, National Institute for Basic Biology, Okazaki 444-8585, Japan*

Contents

* Corresponding author. Tel.: +1-604-666-7926; fax: +1-604-666-3497.
E-mail address: devlinr@pac.dfo-mpo.gc.ca (R.H. Devlin).

PII: S0044-8486(02)00057-1

Abstract

A great deal of information is known regarding the process of sex differentiation in fish, and the mechanisms involved in primary sex determination are now beginning to be defined. A range of gonadal differentiation types have been described for fish, including gonochoristic species possessing purely ovarian or testicular tissues, as well as hermaphroditic species that can initially mature either as males (protandrous) or females (protogynous). Sex determination in fish is a very flexible process with respect to evolutionary patterns observed among genera and families, and within individuals is subject to modification by external factors. These influences can affect the fate of both somatic and germ cells within the primordial gonad, and include the action of genetic, environmental (e.g. temperature), behavioural, and physiological factors. Exogenous sex steroids administered at the time of sex determination can strongly influence the course of sex differentiation in fish, suggesting that they play a critical role in assignment of gonad determination as well as subsequent differentiation. Detailed information is available from fish systems describing the production of sex steroids, as well as the enzymes involved in steroid production. Both estradiol and the maturation hormone 17α, 20β-dihydroxy-4-pregnen-3-one (17α, 20β-DP) are produced by a two-step process involving different cell layers in the gonad, and have effects on the differentiation of gonadal and nongonadal tissues. Gonadal development and differentiation in some fish is also controlled by hormones from the pituitary gland (gonadotropins) that are regulated by release hormones (GnRH) and other neuroendocrine and gonadal factors. Genetic determination of sex in fish can involve monogenic or polygenic systems, with factors located on the autosomes or on sex chromosomes. In the latter case, both male (XY) and female (ZW) heterogametic systems have been described, as well as many subtle variations on these themes. Sex chromosomes are found in approximately 10% of fish species examined, and sex-linked phenotypic traits, and protein and molecular genetic markers have been identified in several fish systems. Some species of fish reproduce gynogenetically, producing all-female populations. Several gene families known to be involved in sex determination in other vertebrates have recently been shown to be similarly involved in fish, suggesting conservation of sex determination pathways. The lability of sex-determination systems in fish makes some species sensitive to environmental pollutants capable of mimicking or disrupting sex hormone actions. Such observations provide important insight into potential impacts from endocrine disruptors, and can provide useful monitoring tools for impacts on aquatic environments.

Keywords: Sexual differentiation; Sex determination; Fish; Gonadal differentiation; Sex steroids; Genetic sex determination; Sex chromosomes; Sex ratios; Polyploidy

---

1. Introduction

It has been over 30 years since the publication of Yamamoto's (1969) seminal review on sex differentiation in fish. Yamamoto's review summarized historical information on gonadal types, hermaphroditism, genetic sex determination, and the influence of sex steroids on sex differentiation, but also provided an important framework for investigation from which followed an explosion of research. In many respects, the broad genetic and physiological principles outlined by Yamamoto remain unchanged, although a great deal of new literature in this area has extended his conclusions to new fish species. On many

issues, additional supporting evidence and revealing exceptions have been obtained, and technological advances occurring in the past 30 years are allowing refinement of the level of study into areas of biochemistry and molecular biology.

The study of sex determination in fish is important for several reasons. There are more than 24,000 species of fish (Nelson, 1994) inhabiting a wide range of aquatic habitats worldwide, providing a rich source of material for academic and applied studies on vertebrate sex determination. Sex determination research in fish has broadened our understanding of this process beyond the specific details found within the group. In particular, the research has provided important insight into the plasticity of the sex-determination process in vertebrates, something that humans do not anthropomorphically anticipate because of the relative stability of sex determination in our species. Focus on or attachment to genetic systems involving X and Y chromosomes must quickly be shed in studies of sex determination in fish due to the diversity of mechanisms utilized, and, by doing so, the researcher can explore alternative regulatory mechanisms that hold important clues for understanding sex determination in general. The biology and ecology of fish is sufficiently diverse to provide unique examples of sex-determination mechanisms, yet they possess many of the same processes and pathways that are used in other vertebrate systems. Because they are amenable to artificial culture and experimental investigation in many cases, fish also provide unique opportunities to investigate and test theoretical concepts of sex determination, ranging from evolutionary mechanisms to biochemical processes.

A perhaps more practical reason for studying sex determination in fish relates to the explosion of human populations in the past two centuries. Food from aquatic systems is an increasingly important component of the human food supply, and finfish comprise the majority of biomass harvested therefrom. Recovery of fish from ocean sources has plateaued in the past decade, forcing demand for aquatic protein to be met through aquaculture production systems. It is imperative that we understand the reproductive biology of harvested species in wild systems to allow effective management and prediction of potential impacts. Similarly, in aquaculture systems, understanding and controlling reproduction is central to the efficient propagation of organisms due to differential growth rates of the sexes and a need for synchronous and reliable maturation.

As integral members of our ecosystem, fish are also becoming increasingly important indicators of environmental health on our planet, with respect to habitat restriction and degradation. In cases where the reproductive capacity of fish populations appear to be compromised, it is necessary to understand the natural population dynamics and life history characteristics of the species involved. Part of this understanding involves knowledge of the strategies and mechanisms involved in reproduction and sex determination to better facilitate investigation into the organismal points of impact.

The present review attempts to summarize research into sex determination in fish from the past 30 years, with a focus on the past two decades. The information presented is focussed on the determination of gonadal sex (the primary controls influencing the course of sex differentiation) and the associated physiological processes that support gonadal development and function. Sex differentiation (the process of gonad development after sex has been determined), sexually influenced characteristics such as behaviour, or morphological or biochemical secondary sex characteristics, are only mentioned briefly when in

context with other observations or when the sex-determination process is labile or can be reversed. The distinction between sex determination and sex differentiation is often difficult since we regularly use criteria of sex differentiation (morphological, cellular, and molecular) to infer whether sex has been determined in a particular direction. For the purposes of this review, sex determination is used to describe the genetic and environmental processes and variables that influence sex differentiation, whereas sex differentiation is used to describe the physical realization of these events in terms of testicular or ovarian development. For additional and historical information and additional views, the reader will be referred in each section to earlier excellent summaries on various aspects of material presented in the current review. In particular, excellent recent reviews discuss aspects of the developmental biology, endocrinology and environmental factors associated with gonadal differentiation and sex differentiation in teleosts (Nakamura et al., 1998; Baroillier et al., 1999). An apology is extended to researchers for omissions from, and terse presentation of, many important studies in this review, primarily resulting from an attempt to provide examples rather than comprehensive coverage of all studies in a particular area. Thus, the reader is encouraged to use the information provided herein as an introduction, from which more in-depth investigations of any chosen area may be undertaken. A visual summary of sex-determination mechanisms utilized by fish is presented in Fig. 1 to assist the reader.

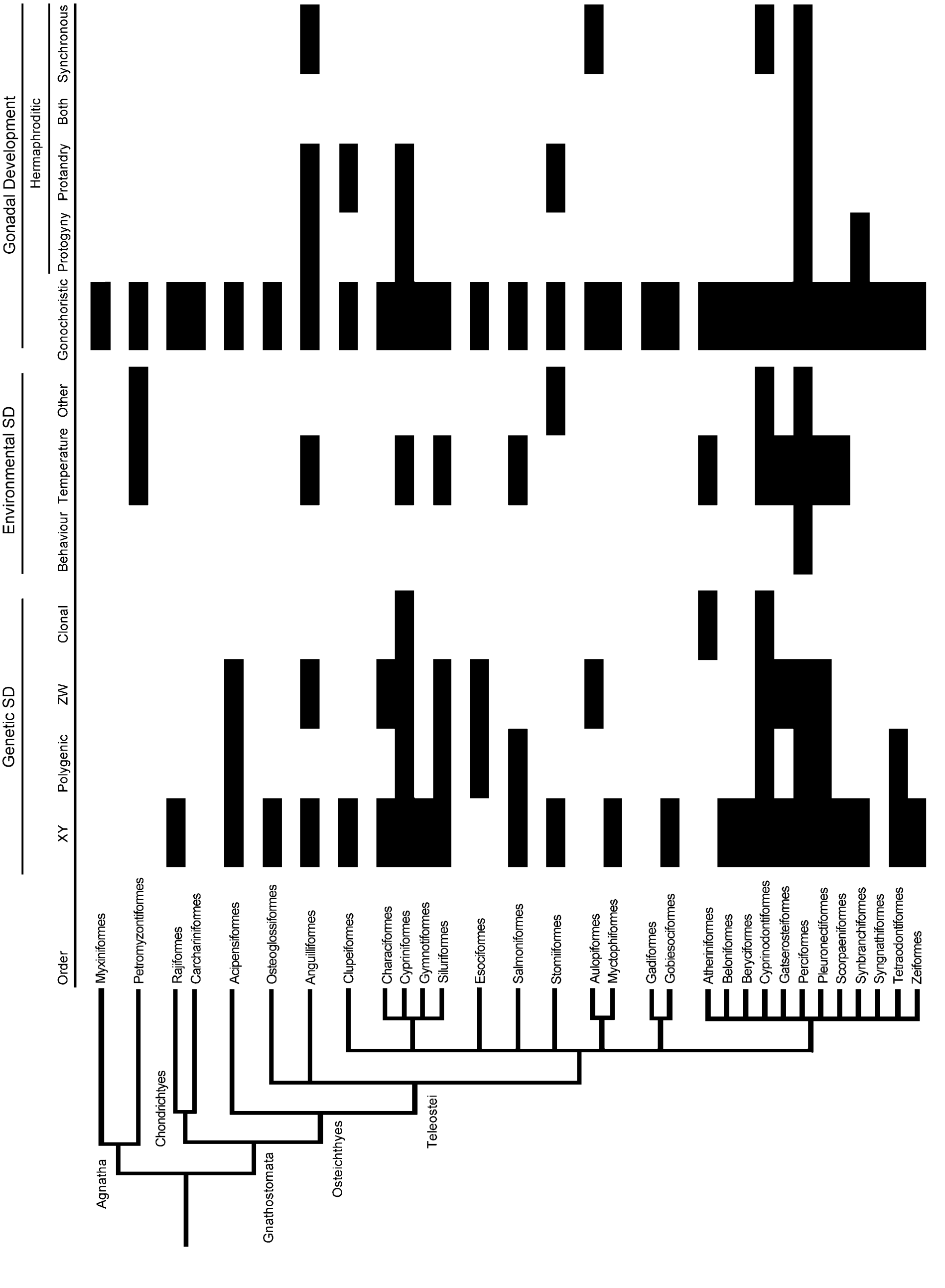
2. Developmental and cellular events

Critical to our understanding of sex-determination processes are studies examining the origin and development of cells and organs involved in the formation of the primordial gonad (Yamamoto, 1969; Chan and Yeung, 1983; Nakamura et al., 1998). Such information includes the descriptive movements of cells during embryonic and subsequent development, interactions among cell types, and differentiation of cells and organs into specialized cell types unique to the testis or ovary. Further, the interactions among germ cells, adjacent somatic cells, and specialized tissues in other organs, all play a role in the development of functional sexes in fish.

Typical of vertebrates, fertilization and egg activation in fish are followed by repeated mitotic cell division to produce a blastula comprised of cells with wide developmental potential (Nilsson and Cloud, 1992). Many fish species possess telolecithal eggs, and meroblastic cleavage distributes cells as a thin blastodisc layer atop a large yolky mass. With subsequent cell movements, development proceeds to the gastrula stage where cell interactions, inductions, and differentiation into germ layers and specialized cell and tissue types ensues. The presumptive gonad region is termed the germinal ridge (Balinsky, 1975), and forms as a longitudinal thickening of mesoderm that protrudes into the coelomic cavity ventral to the developing kidney and lateral to the dorsal mesentery.

2.1. Development of germ cells

The structure of fish gonads is similar to that of other vertebrates, with germ cells and associated supporting somatic cells intermixed. Within both ovary and testis, a clear


Genetic SD
XY
Polygenic
ZW
Clonal
Environmental SD
Behaviour
Temperature
Other
Gonadal Development
Gonochoristic
Hermaphroditic
Protogyny
Protandry
Both
Synchronous
Order
Myxiniformes
Petromyzontiformes
Rajiformes
Carchariniformes
Acipensiformes
Osteoglossiformes
Anguilliformes
Clupeiformes
Characiformes
Cypriniformes
Gymnotiformes
Siluriformes
Esociformes
Salmoniformes
Stomiiformes
Aulopiformes
Myctophiformes
Gadiformes
Gobiesociformes
Atheriniformes
Beloniformes
Beryciformes
Cyprinodontiformes
Gatserosteiformes
Perciformes
Pleuronectiformes
Scorpaeniformes
Synbranchiformes
Syngnathiformes
Tetraodontiformes
Zeiformes
Agnatha
Chondrichtyes
Gnathostomata
Osteichthyes
Teleostei

distinction between germ and somatic cells can be made (particularly at later stages), with the former having the potential to mitotically divide and enter meiosis, and the latter differentiating into associated structural and endocrine cell types. As is the case for many vertebrate and nonvertebrate organisms, the developmental origin of these two cell types also differs in fish.

In many organisms, the unfertilized egg has been shown to contain specialized granular cytoplasm that is often found in vegetal regions of the egg (Wei and Mahowald, 1994), and cells developing around this material remain undifferentiated and physically separated from most early developmental events. These special cells subsequently develop into primordial germ cells (PGCs), which ultimately form gamete-forming cells within the gonad. The cytoplasmic substances responsible for inducing PGC development have not been fully defined as yet, but appear to be specialized maternally deposited RNAs encoded by maternal genes involved in PGC formation. Maternal-effect sterile mutations in *Drosophila* have allowed identification of genes involved in germ-cell development, one of which is a maternal mRNA encoding a DNA helicase (Olsen et al., 1997; Yoon et al., 1997). Granular cytoplasmic inclusions (nuage) have been identified in PGCs of teleosts (Hogan, 1973; Hamaguchi, 1982), implying that unique genetic products are probably also required in fish to allow differentiation of PGCs from other cell types. Indeed, in zebrafish, DNA-helicase RNA (encoded by the *vas* gene) becomes concentrated along cleavage planes at the two-cell stage of embryogenesis, and remains exclusively associated with four cells (putative PGCs) through development to the 1000-cell stage (Yoon et al., 1997). These *vas*-positive cells undergo few divisions, resulting in approximately 30 cells that become associated with the bilateral germinal ridges at the time of somitogenesis. After embryogenesis, *vas* expression in zebrafish (Yoon et al., 1997) and tilapia (Nagahama, 1999) can be detected only in germ cells in the developing gonad (Fig. 2), suggesting that this protein plays an important role in distinguishing germ cells from somatic cells. Expression of reporter genes from homologous *vasa*-like gene promoters in transgenic rainbow trout and medaka has revealed that PGC-specific expression (Fig. 3) also occurs in these species (Yoshizaki et al., 2000; Tanaka et al., 2001). Other indirect evidence in fish suggesting genetic control of PGC formation and/or maintenance has been presented for rainbow trout, where an inbred family possessed gonadal tissue but completely lacked any germ-cell development (Herman and Kincaid, 1986). Similarly, different genetic lines of carps have been found to possess different numbers of PGCs, which ultimately affects germ cell development and fecundity (Ryazantseva and Sakun, 1980; Bieniarz, 1986).

Once formed, PGCs remain closely associated with endodermal tissues (near yolk sac epithelium in some species) and migrate via the dorsal gut mesentery to the region of presumptive gonad. Gonadal development has been carefully studied in medaka, including the origin and migration of PGCs (Satoh and Egami, 1972; Hamaguchi,

Fig. 1. Summary of endogenous mechanisms and external variables influencing different types of sex differentiation utilized by major orders of fish. Under Gonadal Development, “Both” indicates protogyny and protandry are observed in the same species.

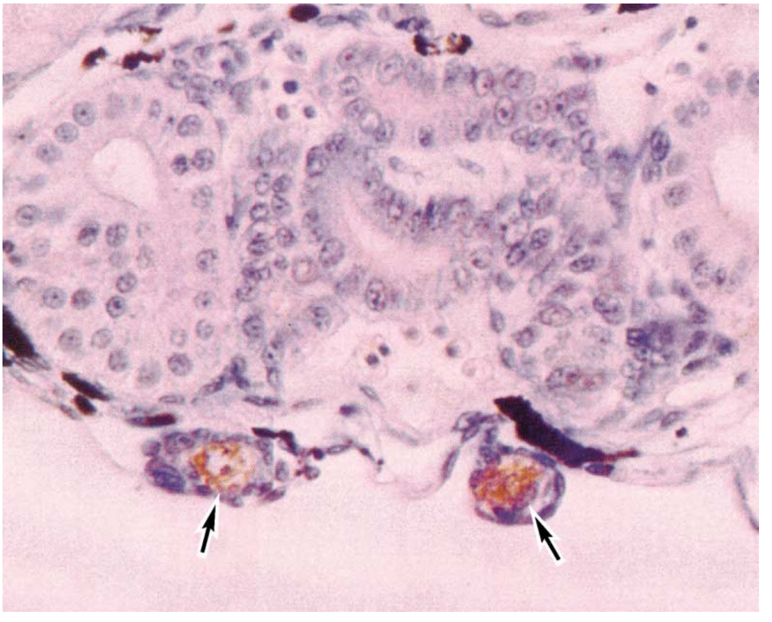


Fig. 2. Vasa protein (arrows) detected by immunohistochemistry in tilapia gonad 3 days posthatching. Vasa is located only in germ cells of both sexes. (Unpublished, provided by T. Kobayashi).

1982, 1992). PGCs have a distinct ultrastructure, and can be first identified in the subendodermal space of the syncytial periblast, from where they subsequently move to the subectodermal space and then mesoderm. PGCs are surrounded by somatomesodermal cells, and passively transferred to the germinal ridge via morphogenetic movements of mesoderm and cell migratory activities (Hamaguchi, 1992). PGCs have been identified in the dorsal mesentery along this route (mesenchymal tissue between the alimentary tract and the dorsal body wall) in *Ditrema temmincki* (Lee and Lee, 1996) and the rockfish *Sebastes schlegeli* (Lee et al., 1996), and between the gut and mesonephric duct in tilapia species (Yoshikawa and Oguri, 1978; Zhu, 1987) and flounder *Paralichthys olivaceus* (Tanaka, 1987). These studies indicate that PGC migration in fish occurs via the dorsal mesentery as in other vertebrates, although some evidence exists in carps that PGC migration can occur via different pathways (Bieniarz, 1986). In most cases, PGCs appear to migrate directly to gonadal tissues, but evidence has also been presented for sturgeon that PGCs can migrate to other organs such as liver (Romanov and Altuf'ev, 1992, 1993). The origin, migration or development of PGCs and other gonadal tissues have been described in several other teleosts, including: *Platypoecilius maculates* (Wolf, 1931), *Micropterus salmoides* (Johnston, 1951), *Perca fluviatilis* (Mezhnin, 1978; Zelenkov, 1982), *Salvelinus leucomaenis* (Nakamura, 1982), *Oncorhynchus mykiss* (Takashima et al., 1980; Van Den Hurk and Slof, 1981; Lebrun et al., 1982), *Neogobius melanostomus* (Moiseyeva, 1983), *Barbus conchonius* (Timmermans and Taverne, 1989), *Cyprinus carpio* (Par-

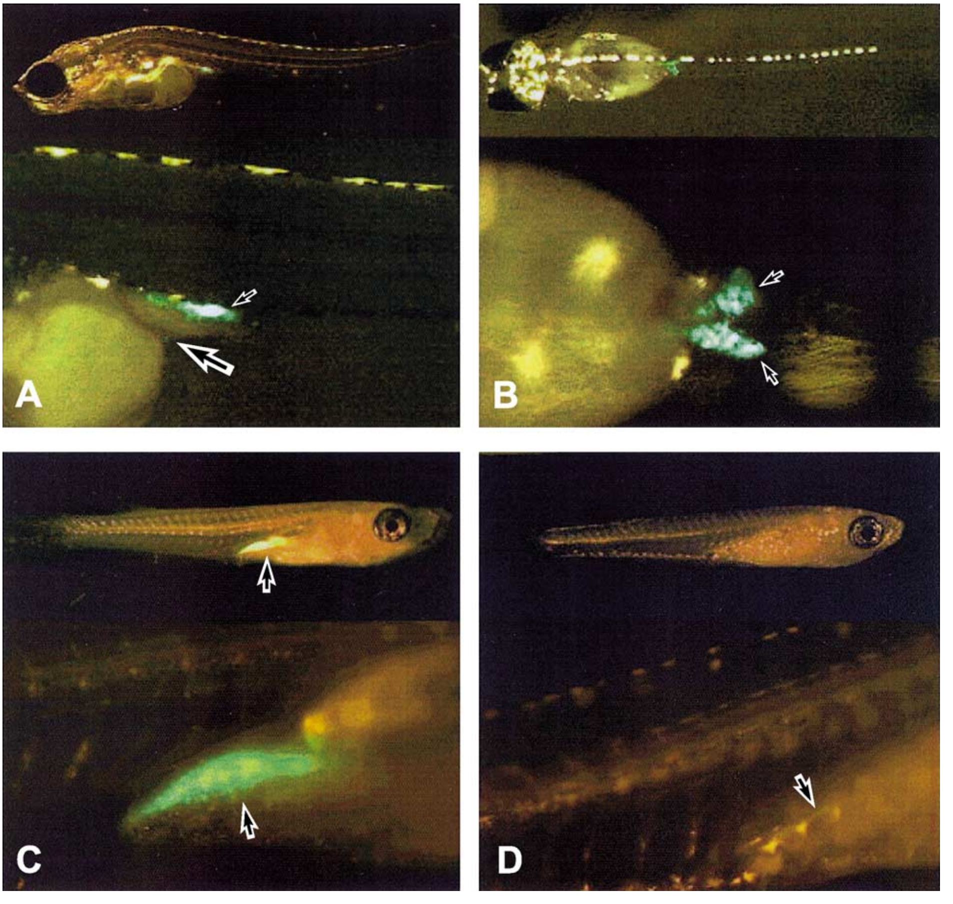


Fig. 3. Localization of GFP expression from a vasa promoter in transgenic whole female medaka. *Vasa* promoter activity is found only in germ cells. (A) Lateral, and (B) ventral views. Small arrow indicates location of *vasa* expression in ovary at hatching. Note ectopic germ cell ventral to the ovary on the yolk surface (large arrow). (C) Female and (D) male at 3 weeks of age. Arrows indicate strong *vasa* expression in ovary, whereas expression in males is limited to a thin streak associated with the testis. (From Tanaka et al., 2001).

mentier and Timmermans, 1985; Shelton et al., 1995), *Acipenser gueldenstaedti* (Akhundov and Fedorov, 1990), *Corregonus laveratus* (Dlugosz and Demska-Zakes, 1990), *Esox masquinongy* (Lin et al., 1997), and *Salaria pavo* (Patzner and Kaurin, 1997).

PGCs in undetermined gonads, testis, and ovaries also possess very characteristic cytological features (Bruslé and Bruslé, 1978a,b), and share unique antigenic sites between the sexes that are absent from somatic cells (Parmentier et al., 1984; Parmentier and Timmermans, 1985; Van Winkoop and Timmermans, 1992). These feature are consistent with the idea that PGCs are similar in the two sexes, and that they remain undifferentiated

and undetermined until exposed to hormonal and other influences from the developing gonad, which transforms them into spermatogonia or oogonia (see below).

2.2. Early gonadal development

Somatic cells of the presumptive gonad have an embryological origin distinct from PGCs. The external somatic layer of the developing gonad is derived from the genital ridge epithelium, but some somatic cells are probably also derived by the invasion of mesenchyme (similar to the primitive sex chords in mammals). However, unlike other vertebrates, no clear indication of medullary tissue can be found in the teleost gonad (Guraya, 1994). Prior to differentiation, all somatic cells appear to be derived from a cortex epithelial layer, and are similar in presumptive males and females. Following migration of PGCs into the germinal ridge, cell division occurs to form oogonia and spermatogonia, and differentiation and migration of somatic cells is initiated concomitant with this process. In mammals, differences in cell migration patterns apparent between the sexes are directed by the sex-determination locus, *Sry*, whose action in somatic cells of the early gonad causes active recruitment of mesonephric cells into the gonad in males (Brennan et al., 1998; Koopman, 1999). Subsequently, in the testis, the somatic cells (surrounding PGCs) differentiate into seminiferous tubules and supporting connective tissue, and into cells similar to Leydig and Sertoli cells found in mammals (Da Cruz-Huefling and Da Cruz-Landim, 1984; Pudney and Callard, 1984; Van Vuren and Soley, 1990). In Japanese eels, evidence has been presented indicating that physical proximity between germ and somatic cells is required for functional gonad development (Miura et al., 1996).

For ovarian development, the somatic cells and PGCs begin to differentiate to form follicles, comprised of oocytes surrounded by an inner granulosa and outer thecal layer (Nagahama et al., 1982). In most species, ovarian development in females is first detectable with the proliferation of somatic cells and oogonia and early oocyte differentiation, which is then followed by the formation of the ovarian cavity. Testicular development usually occurs later than ovarian differentiation, some weeks or months after the onset of gonad development in females of some species (Guraya, 1994; Nakamura et al., 1998). In salmonids, at the time of hatching, primordial gonads can be identified as paramedian ridges on the dorsal coelomic wall (Nakamura et al., 1974). PGCs can be identified in the germinal ridge (Nakamura et al., 1974; Foyle, 1993), and a clear distinction between epithelial and stromal cells can be made. However, at this time, histological evidence does not reveal distinction of male or female gonadal cell types, which would indicate that sex determination has occurred and that sex differentiation is proceeding. The first evidence of change from an indifferent gonad in salmonids is observed in females, with the differentiation of oocytes in prophase at 3 weeks posthatching (Nakamura and Nagahama, 1993). Similarly, in coho salmon, differentiation of ovarian tissue has been observed as soon as 27 days posthatching (Piferrer and Donaldson, 1989), whereas differentiation of testicular tissue occurs several weeks later.

Gonads usually develop bilaterally, but may be single or fuse in some fish species (e.g. Rasotto, 1992; Taylor and Burr, 1997); in the mud eel, *Monopterus albus*, it has been reported that only the left gonad develops (Mei et al., 1993).

3. Gonadal differentiation

Gonadal differentiation can take many forms in teleosts, ranging from the familiar case where individuals directly develop and finally possess only testis or ovaries at sexual maturation, to synchronous hermaphroditic species that contain functional male and female gonadal tissues at the same time. Between these extreme types, there is a remarkable array of strategies involving sex reversal and sex ratio bias in populations, arising from frequency or temporal differences in the development of testis or ovary from hermaphroditic gonads. It is beyond the scope of this review to cover in depth the many different forms of sexuality that exist among hermaphroditic fishes. Only selected recent examples are provided for illustration, and the reader is directed to a summary of hermaphroditic fish in Appendix A and other reviews (Yamamoto, 1969; Ross, 1990; Shapiro, 1990) for more information.

3.1. Gonochoristic species

Many teleosts are gonochorists, where individuals develop only as males or females, and remain the same sex throughout their life spans. It is important to note that the final sexual states of maturity achieved in fish may not reflect the initial gonadal developmental pathway taken. For example, gonochorists may develop directly as males or females, or they may develop with gonads that are initially hermaphroditic but that subsequently resolve into only functional ovaries or testis. With respect to mechanisms of sex determination, this latter group may be more like functional hermaphrodites than direct gonochorists, having simply lost the ability to retain both gonadal tissue types through to sexual maturity.

It is apparent that different strategies of early gonadal development are employed among gonochoristic species, and Yamamoto (1969) has summarized historical data that identified two major types. In *differentiated* gonochoristic species, also termed primary gonochorism (Atz, 1964), early gonad development proceeds from an indifferent gonad directly to ovary or testis [e.g. *Oncorhynchus kistuch* (Piferrer and Donaldson, 1989), *Abramis brama* (Talikina, 1995), *E. masquinongy* (Lin et al., 1997), *S. schlegeli* (Lee et al., 1996), *Dicentrarchus labrax* (Blázquez et al., 1998a), and *C. carpio* (Komen et al., 1992b)]. An alternative mode of gonadal development is found in *undifferentiated* species where all individuals initially develop ovarian tissue. In the teleosts *Danio rerio* (zebrafish) and *Barbus tetrazona*, all gonads initially develop as ovaries, but in approximately half of the population, ovarian tissue subsequently degenerates and the gonad is invaded by additional somatic cells (Takahashi, 1977; Takahashi and Shimizu, 1983). Masculinization of the gonad then proceeds to produce an initially intersexual gonad that ultimately resolves into a normal testis.

Some gonochoristic fish species go through a period where all gonads are initially intersexual prior to differentiation into either testis or ovary [e.g. *Cheimerius nufar* (Coetzee, 1983), *Epinephelus striatus* (Sadovy and Colin, 1995), or *Gramma loreto* (Asoh and Shapiro, 1997)]. Such species, termed secondary gonochorists (Atz, 1964), may appear at first examination to be developing as functional hermaphrodites, but a complete analysis of all developmental stages of gonadal development is usually required to discern the exact mode of gonadal developmental (rather than a snapshot of limited stages).

Where most individuals mature as only one sex (indicating the species is primarily gonochoristic), rare individuals can be found that have undergone sex change or remained as intersexes until maturity (Sadovy and Colin, 1995). In European and Japanese eels (*Anguilla anguilla* and *A. japonica*), juvenile individuals initially possess a bipotential intersexual gonad that can develop directly into an ovary or testis (Tesch, 1977; Colombo and Grandi, 1990, 1995, 1996; Beullens et al., 1997), although juvenile hermaphroditism in *A. anguilla* has not been observed in some studies (Bieniarz et al., 1981). In the Agnathan *Eptatretus stouti*, all juveniles are found to contain ovarian development in anterior gonadal regions, whereas the posterior initially remains undifferentiated. Later, the posterior region can develop into a testis, correlated with the regression of anterior ovarian tissue in some cases (Gorbman, 1990). In this hagfish species, and also some lamprey (Yamamoto, 1969), some adult individuals develop with both male and female germ tissue in their gonads. Similarly, juvenile presumptive testis (consisting largely of undifferentiated germ cells) of lamprey *Mordacia mordax* contain ovarian cell types that ultimately undergo atresia as spermatogenesis progresses (Hardisty et al., 1992). This phenomenon has been examined in detail in *Petromyzon marinus* by examining gonadal biopsies from early larvae and subsequently examining gonadal differentiation from the same animal at later stages (Lowartz and Beamish, 2000). Larvae that initially possess ovaries or atypical gonads (with mixtures of oocytes and germ cells) can ultimately resolve into testis by oocyte atresia. In some cases, complete sex reversal was detected, indicating that primary sex differentiation in this species is not definitive. In the normally gonochoristic *Xiphophorus helleri*, hermaphroditism has been observed where three individuals in one strain have been found to switch from female to male (Lodi, 1979). This species possesses a very plastic mode of sex differentiation (see Section 5.5.1), suggesting that sex reversal events in this case may arise by the extreme action of normal regulatory processes.

3.2. Normal hermaphrodites

As mentioned above, gonochorists are species where individuals develop as males or females and do not reverse sex at any time during their lifespan. In contrast, hermaphrodite fish can produce mature male and female gametes at some time in their lives, and thus are a very interesting group among the vertebrates for the study of sex differentiation. Several different types of hermaphrodites have been described and the terminology for the different types has been described in detail (Atz, 1964; Sadovy and Shapiro, 1987). Gonadal types should ideally classified based on functional criteria (i.e. the types of sexually mature reproductive states that an individual may attain in its lifespan) rather than on gonadal developmental pathways or structures observed at a particular life-history stage (Reinboth, 1975; Chan and Yeung, 1983; Sadovy and Shapiro, 1987). Importantly, hermaphrodites are differentiated from those gonochoristic species that possess both sexes of immature gametes in a single gonad at some point in development, but that ultimately resolve into a single stable sex per individual [termed rudimentary hermaphrodites (Buxton and Garratt, 1990)]. A very large literature exists describing hermaphrodite fish gonad development, behaviour, and mating strategies, and only examples are provided below to illustrate the various forms currently described. Sex reversal in hermaphrodites

may take months to accomplish, or as little as days to weeks in others (Sadovy and Shapiro, 1987).

In functional hermaphrodites, at least some individuals within a population will sexually mature as both sexes in their lifespan. However, our understanding of germ cell development in hermaphrodites is not well understood (Sadovy and Shapiro, 1987), and it is not clear whether sex reversal represents stimulation of development of previously quiescent, but already determined, germ cells of the second sex, or whether new primary (or redefinition of) germ cell determination is occurring. It is possible that hermaphroditism and sex reversal is regulated at the gonadal level, with individuals possessing mixtures of male and female determined germ cells that are stimulated to proliferate and differentiate under differing conditions (see below).

Synchronous hermaphrodites (also termed simultaneous hermaphrodites) produce both male and female gametes at the same time, of which some species are capable of alternating between sperm or egg delivery [e.g. *Serranus* sp. (Pressley, 1981; Petersen, 1990; Oliver, 1991, 1997) or Gobiidae (Cole, 1990; St. Mary, 1998)], and, remarkably, some (*Rivulus marmoratus*) that are even capable of internal self fertilization (Soto et al., 1992; Cole and Noakes, 1997). In sequential hermaphrodites (Sadovy and Shapiro, 1987), individual fish first produce one gamete type, then reverse sex and produce the other type in a subsequent spawning cycle. Sequential hermaphrodites are classified as protandrous if they mature first as males [e.g. *Sparus aurata* (Buslé Sicard and Fourcault, 1997) or *Amphiprion* sp. (Hattori, 1991; Hattori and Yanagisawa, 1991a; Godwin et al., 1996)], or protogynous if development first occurs as female (e.g. Shapiro, 1980; Shapiro and Rasotto, 1993; Shapiro et al., 1993a; Reinboth and Buslé Sicard, 1997). Some few species have been characterized that can reverse sex in both directions (see below), and some species display hermaphroditic and gonochoristic subpopulations (e.g. Lodi, 1980). Among protogynous species, monandrous forms have males developing only from fish that had previously matured as female (e.g. Ferreira, 1993; Gillanders, 1995), whereas diandrous species have two sources of males, derived either from immature bisexual stages or by sex reversal of females that have previously sexually matured (Gordo, 1995). The existence of digyny in fish, although rare, has been reported for *Lates calcarifer* (Moore, 1979).

These different forms of hermaphroditism have now been observed in at least 25 families of fish, indicating that these modes of sexual differentiation are widespread among teleosts (see Appendix A for a partial list of hermaphroditic fish species). Stable systems of sex determination that involve hermaphroditism are not anticipated where strong genetic sex-determination systems are functioning (see Section 5). Indeed, among 259 species displaying some form of hermaphroditism (Appendix A), only seven genera and four species have been found to possess sex chromosomes in some populations and/or individuals (compare Appendices A and B). Within each of several families (Gobiidae, Muraenidae, Pomacentridae, Serranidae, and Sparidae), both protandry and protogyny can be found, as well as some gonochoristic and synchronous hermaphroditic species (Buxton and Garratt, 1990; Cody and Bortone, 1992; Fishelson, 1992). In Serranidae, both synchronous and protogynous forms are found, whereas within the Gobiidae, some species are gonochoristic and some are protogynous, and still others capable of switching sex in either direction have been discovered (Robertson and Justines, 1982; Cole, 1990;

Kuwamura et al., 1994; Nakashima et al., 1995; Munday et al., 1998). Similarly, some Japanese hawkfish (*Cirrhitichthys aureus*) individuals are capable of switching sex in both directions (Kobayashi and Suzuki, 1992). For *R. marmoratus*, most individuals are synchronous hermaphrodites, but some fish can switch from being hermaphrodites to males, whereas others can develop as primary males (Soto et al., 1992; Lubinski et al., 1995). This plasticity in sexual differentiation among closely related fish species suggests that different reproductive strategies may be able to evolve quite easily via subtle alterations in normal pathways that regulate the timing and course of testicular and ovarian development in embryos, juveniles, and adults.

The structure of gonads in hermaphrodites can be predominantly ovarian or predominantly testicular at different stages (e.g. Yamamoto, 1969; Zohar et al., 1978; Chan and Yeung, 1983; Sadovy and Shapiro, 1987), or in others, only minor indications of hermaphroditism can be detected histologically [e.g. in both sexes of wrasse *Pseudolabrus gymnogenis* (Labridae) (McPherson, 1977)]. Male and female germ cells may be organized in three major ways: Delimited gonads possess male and female germ tissues that are separated by a membrane, undelimited gonads that either have separate male and female tissue not separated by connective tissue, or undelimited gonads possessing interspersed gametes (Sadovy and Shapiro, 1987). Male and female germ tissues can be in very close proximity in some species, such as in the protandric *Amphiprion frenatus*, where developing oocytes are found separated from testicular tissues only by respective somatic cell layers in males (Bruslé Sicard and Reinboth, 1990). In others (e.g. *Rhabdosargus sarba*), discrete separation of male and female tissues by significant amounts of connective tissue is observed (Yeung and Chan, 1987d). In the sequential hermaphroditic Gobiidae genus *Lythrypnus*, five species all possess two spatial points (dorsal and ventral) of testicular development in the ovary during sex change, and this pattern differs from the development of testicular tissue (single point, partitioned, or interspersed) observed in other Gobiidae genera (St. Mary, 1998).

In protogynous species, no indication of testicular tissue may be apparent in young individuals of *Dascyllus reticulatus* (Schwarz and Smith, 1990), but in the wrasse *Thalassoma bifasciatum*, undifferentiated gonads initially develop as ovaries, which, in females, continue that course of differentiation (Shapiro and Rasotto, 1993). However, in primary males of this latter species, ovarian development is suppressed and testis develop, whereas secondary males develop testicular tissue in females after maturity (Shapiro and Rasotto, 1993). Other features often associated with protogynous sex reversal is the presence of a remnant ovarian cavity in the testis, although this can also be detected in some gonochoristic species that develop from a bisexual condition (Sadovy and Colin, 1995) and can thus reflect events occurring in very early gonadal differentiation (i.e. whether the species is differentiated or undifferentiated). The progression from ovary to testis in the protogynous *Thalassoma duperrey* (Morrey et al., 1998) is shown in Fig. 4.

In the protogynous Serranid *Plectropomus maculatus*, sperm ducts do not form initially, but rather sperm is collected in intragonadal sinuses that initially form in the peripheral gonadal musculature (Ferreira, 1993). Similarly, in protandrous species, ducts within new ovarian tissue develop to allow delivery of ova (Godwin, 1994b). In synchronous species, two duct systems are apparent (Abd-el-Aziz and Ramadan, 1990). Duct formation and

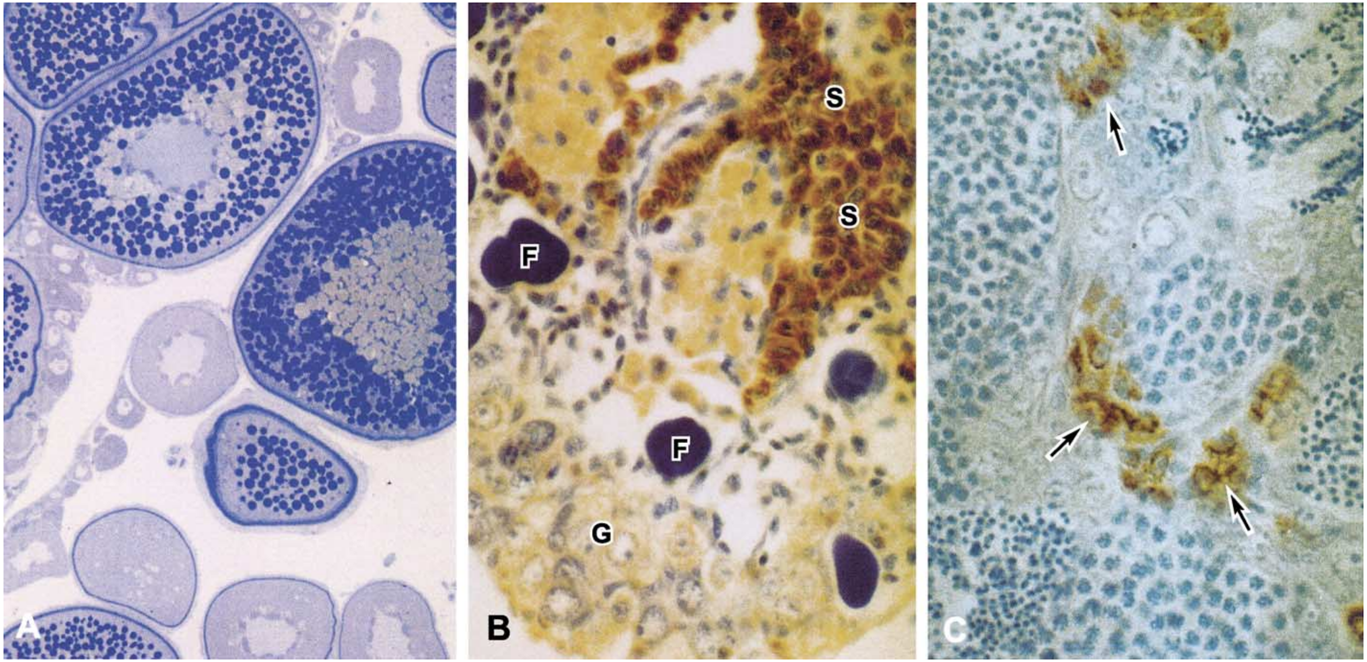


Fig. 4. Photographs of gonads during sex change in the protogynous hermaphrodite wrasse *Thalassoma dupperrey*. (A) An ovary before sex change showing vitellogenic follicles. (B) A gonad during sex change showing atretic follicles (F), putative spermatogonia (G) and steroid-producing cells (S). Steroid-producing cells are positive to *P*450 cholesterol side chain cleavage enzyme (*P*450scc) immunostaining. (C) A testis after sex change showing germ cells during various stages of spermatogenesis and *P*450scc positive steroid-producing cells (Leydig cells) (arrows). (Morrey et al., 1998).

variations seen in hermaphrodites have been described in detail (Sadovy and Shapiro, 1987).

Hermaphroditism and sex change has evolved to provide individual members of fish populations with maximum reproductive output (e.g. by increasing numbers or quality of eggs produced, enhancing competitive fertilization by increasing milt volume or controlling harems, or enhancing survival of progeny under parental care). The diversity of sex change (Fig. 1) observed among different fish at all phylogenetic levels is testimony to the large number of habitats they utilize, the diversity of life histories employed, and the complexity of their demographic interactions.

3.3. Abnormal hermaphrodites and intersexes

In gonochoristic species, which normally only possess either ovarian or testicular tissue in their gonads, examples of hermaphroditism or spontaneous sex reversal are very rare (Atz, 1964). Such exceptional intersex fish, termed abnormal hermaphrodites (Atz, 1964) or intersexes (Yamamoto, 1969), are often observed by chance in field or laboratory surveys where gonad developmental stage or sex ratio is being examined, and thus probably provide an unbiased minimum approximation for the spontaneous frequency of hermaphroditism in these populations. The phenotypes of abnormal hermaphrodites range from mixed male and female gonadal tissue represented by a very few cells of one type among an otherwise complete gonad of the opposite sex to very large portions of the gonad being allocated to testis and to ovary. Intersexes have been discovered at stages prior to sexual maturity, or in some cases, when functional gametes of both sexes are being produced.

More than 100 historical examples of abnormal hermaphroditism have been reported (see Atz, 1964; Dawson and Heal, 1977; Honma, 1980). More recently, single specimens of hermaphrodites have been identified in several other teleosts: *P. fluviatilis*, with both functional sperm and developing eggs (Jellyman, 1976); *Salvelinus alpinus*, with an ovarian lobe in a male (Fraser, 1997); *Menidia beryllina*, where 10% of the gonad was testis in a female (Yan, 1984); *Micropogonias furneri*, with testicular structures and vitellogenic oocytes in what otherwise appeared to be an ovary (Macchi and Christiansen, 1994); *Heterandria formosa*, with a gonad showing signs of vitellogenic oocytes and spermatozoa, with the ovarian regions dominant (Riehl, 1991); *Fundulus diaphanus*, with both mature sperm and eggs present (Porter and Fivizzani, 1983); *Sardinops sagax*, with both ova and spermatocytes (Herrera et al., 1991); *Salmo trutta*, producing functional eggs and sperm (O'Farrell and Pierce, 1989); and *Oncorhynchus keta*, where four individuals have been identified that contain testicular and ovarian tissue (Hikita and Hashimoto, 1978; Honma, 1980; Devlin, unpublished observations). Similarly, in a normally gonochoristic cartilaginous fish, *Centroscyllium fabricii*, 4 among 2600 individuals examined contained both ovary and testis (Yano, 1995).

Histological evidence for hermaphroditism in juveniles has been found in *Odontethes bonariensis*, where a few oocytes at the diplotene stage have been identified in a testis (Strüssmann et al., 1996a), and an intersex condition has also been identified in the roach, *Rutilus rutilus*. Intersexes were identified at very low (0.02) frequency in wild populations (Schultz, 1996), and in the same species, a testis had some oocytes under

microscopic examination (Jafri and Ensor, 1979). Similarly, oocytes (termed testis–ova) are apparent in the testis of *Tilapia zillii* (Yoshikawa and Oguri, 1978), *Channa punctatus* (Srivastava and Singh, 1989) and *D. labrax* (Roblin and Bruslé, 1983), but these are usually smaller than the ova found within regular ovaries, and caution is required in definitively identifying such cells as true oocytes (see Yamamoto, 1969 for discussion and other examples).

The causes of these examples of rare abnormal hermaphroditism are unknown. Environmental effects (e.g. temperature or xenobiotics, see Section 6) in species with labile sex-determination mechanisms may be responsible for some cases of sexual ambiguity, or normal variances that exist in endogenous sex-determination physiology may be the cause (e.g. variable hormone production or reception). For example, altering sex-steroid levels in salmonids at the time of sex determination can cause development of hermaphroditic individuals with functional sperm and ova (Jalabert et al., 1975; Chevassus et al., 1988). Alternatively, germline mutations may occasionally occur in populations that alter sex-determination processes in descendants (e.g. Komen et al., 1992a). Genetically mosaic individuals may also arise through mutations occurring in cells during development, altering the generation or interpretation of sex-determination signals for a subset of cells within the gonad. In this regard, two separate examples of hermaphrodite chum salmon have been observed (unpublished observations), in one case the genetic sex was male based on the use of Y-chromosomal DNA markers (see Section 5.4.3). In this hermaphrodite, gametes collected from the feminized portion of the gonad segregated the sex-reversing mutation to the next generation.

Although these examples of abnormal hermaphroditism occur infrequently in populations, they may provide opportunities for species to explore new modes of sex differentiation, and, for the investigator, can allow insight into the evolutionary mechanisms that modify and/or stabilize these processes.

3.4. Stability of sex determination in gonochorists

In mammalian systems, once sex has been determined, gonadal differentiation usually proceeds down a single developmental pathway to yield fully differentiated testes or ovaries (Hawkins, 1994; Capel, 1998). In fish, there are many exceptions to this rule, where development of the gonad may be influenced by fluctuations in intrinsic factors such as growth or behaviour, or by extrinsic environmental factors such as temperature, endocrine hormones or pollution (see Sections 3.2 and 6). In gonochoristic species, many influences on gonadal function occurring after sex determination are associated with impairment of reproduction (see Sections 6.1 and 6.4). For example, treatments of rainbow trout with testosterone or estrogen after sex differentiation has been initiated can arrest gametogenesis, causing oogenesis to be arrested in females and regression of the testis in males (Billard et al., 1982). Similarly, dietary treatment of female rainbow trout with 17α-methyltestosterone after the normal time of sex determination can induce testicular development, but this effect was transitory, and masculinization effects were reduced when the fish were examined 6 months later (Olito and Brock, 1991).

In other cases, definitive evidence has been obtained that gonadal differentiation can be reversed, particularly in hermaphroditic species, but also in gonochorists. However, for

normal hermaphrodites, it is unclear whether sex reversal occurs by redetermination of germ and associated cells, or whether replacement of one gonad cell type by another occurs by deterioration and proliferation of respective existing previously determined primordia (see below). In gonochoristic common carp possessing differentiated ovaries, treatment with 17α-methyltestosterone can cause regression of ovarian tissue, and induction of testes, which produce functional, genetically female sperm (Gomelsky, 1985). Estradiol treatment of gonadally undifferentiated eels (*A. anguilla*) causes feminization, as is observed for many species (see Section 6.1). However, when treatment was delayed until after morphological differentiation of testis had occurred (normally 95% male in this strain), sex reversal could still occur and result in 44% females (Andersen et al., 1996). Similar effects are observed with barfin flounder *Verasper moseri*, where treatment with high doses of estradiol can feminize males even after testicular development had been initiated (Mori et al., 1995), and exposure of mature male carp to the endocrine disrupter 4-*tert*-pentylphenol or estradiol can result in degeneration of the testis, and the latter compound can induce an ovotestis (Gimeno et al., 1998b).

In sex-reversing fish, the protogynous grouper *Epinephelus suillus* can be precociously masculinized by 17α-methyltestosterone treatment, but such fish revert back to females upon withdrawal of the hormone, and can actually begin to produce ova again (Tan-Fermin, 1992). In the protandrous black porgy, *Acanthopagrus schlegeli*, estradiol treatment can, at high doses, induce regression of testicular tissue and stimulate development of ovarian tissue (Chang et al., 1995a,c). Unlike for gonochoristic species, interpretation of these experiments with sex-changing fish is difficult because of their normal process of gonadal development. However, steroid treatments certainly can influence the rate or frequency of this natural sex-changing process in hermaphrodites (see Section 4.8).

Of considerable interest is the source of cells involved in the redifferentiation of gonads. It is not clear whether individual differentiated cells (spermatogonia or oogonia) are able to switch developmental fates during sex reversal, or whether undetermined germ cells within the gonad are recruited to initiate a new course of differentiation. Classical experiments in *Betta splendens* (Noble and Kumpf, 1937 and Kaiser and Schmidt, 1951 described in Yamamoto, 1969; more recently reexamined by Lowe and Larkin, 1975) showed that over 40% of females that had been ovariectomized could sex-reverse into functional males. These experiments indicate that redetermination and redevelopment of the gonad from residual tissue (gonadal duct) can proceed in a direction opposite to the original genetic sex. In genetically female goldfish, ovariectomy of fish normally results in sterilization, but treatment with 11-ketotestosterone can induce testicular development, again indicating that sexual differentiation remains labile, perhaps throughout the life of the animal (Kobayashi et al., 1991). Support for this concept also comes from the observation that PGCs can be found in differentiated ovaries, testes, and ovatestes of *Liza aurata*, *Serranus hepatus*, *Coris julis* (Bruslé, 1988) and *Xiphorphorus* sp. (Flores and Burns, 1993). The presence of PGCs at many stages of gonadogenesis suggests that potential for sex reversal and redifferentiation may be maintained throughout the reproductive lifespan of many fish.

4. Endocrine and molecular control of sex differentiation

The extraordinary migration of PGCs from yolk-sac endoderm to within the gonadal anlagen indicates that even early events associated with sex determination and gonadogenesis require interactions among cell types. Subsequent growth and differentiation of the gonad also involves communication with nonadjacent tissues via endocrine controls that are distinct for the two sexual phenotypes. Endocrine control of sex differentiation involves a complex interplay between the brain and gonad through the production of pituitary-derived gonadotropins and steroids produced in the gonad and brain (Bieniarz and Epler, 1992; Nagahama, 1994). Sex steroids have local, direct effects on germ-cell development, but also act as endocrine hormones to influence other cell types and organs involved in sex differentiation. This multilevel control is very complex, and involves a multitude of biochemical, neurological, and physiological pathways to provide the necessary plasticity for gonadal development to proceed in context with intrinsic and environmental factors. This complexity also provides many levels at which reproduction can be disrupted (see Section 6).

Although nonsteroidal hormones are synthesized in, or act on, gonadal tissues (e.g. somatotropins and insulin-like growth factors, (Van der Kraak et al., 1990; Duan et al., 1993; Kagawa et al., 1995; Maestro et al., 1995; Le Gac et al., 1996), the most intensively investigated hormones are the sex steroids. Estradiol-17β is found at much higher levels in females than males, and is believed to be the major sex steroid responsible for inducing and maintaining ovarian development (Yamamoto, 1969). Both testosterone and 11-ketotestosterone are found in males, the latter being the major androgen responsible for testicular development (Jiang et al., 1996; Miura et al., 1996; Nagahama, 1999). In addition, maturation steroids such as 17α, 20β-dihydroxy-4-pregnen-3-one (17α, 20β-DP), as well as other direct precursors of these hormones (from pregnenolone) have been studied in fish. A variety of nonclassical sex steroids are also produced in fish gonads (Kime, 1993).

4.1. Enzymology of steroid production in fish

As in other vertebrates, a complex series of enzymes are responsible for the biosynthesis of sex steroids in fish (Nagahama, 1994). Specific genes involved in steroid biosynthesis are differentially expressed in the somatic cells of testis and ovary (Omura and Morohashi, 1995; Nakamura et al., 1998), which results in the production of an array of sex steroids. The pathway initiates with the synthesis of the steroid precursor pregnenolone via side-chain-cleavage of cholesterol by *P*450scc, which is followed by the production of progesterone, 17α-hydroxypregnenolone, 17α-hydroxyprogesterone, dehydroepiandrosterone, and androstenedione occurring via 17α-hydroxylase and C17, 20 lyase activities in combination with 3β-hydroxysteroid dehydrogenase (Nagahama, 1994, 1999). In medaka ovary (Kobayashi et al., 1996a), progesterone is not detected at any stage of maturation, indicating that conversion of pregnenolone to 17α-hydroxypregnenolone is preferred. Estradiol's precursor testosterone can be produced both from androstenedione with 17β-hydroxysteroid dehydrogenase activity, or from dehydroepiandrosterone and 5-androsten-3β, 17β-diol via 17β-hydroxysteroid dehydrogenase and 3β-hydroxysteroid dehydrogenase activities. Results indicate that the former pathway (via

17α-hydroxyprogesterone) is primarily utilized in vitellogenic ovaries (Kobayashi et al., 1996a).

Testicular tissues of common carp cultured in vitro convert pregnenolone and androstenedione primarily to 11β-hydroxyandrostenedione, whereas in vivo, the dominant circulating androgen is 11-ketotestosterone (Cavaco et al., 1997). These results imply that further conversion of 11β-hydroxyandrostenedione to 11-ketotestosterone by 11β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase may occur, at least in part, in nontesticular tissues such as blood (Schulz, 1986; Schulz and Bluem, 1991). Metabolism of testosterone and androstenedione has also been shown in stickleback kidney tissue (Borg et al., 1992). In ovary, thecal steroids (mainly testosterone), are made available to granulosa cells (see below) where the enzyme aromatase (*P*450aro) is expressed, resulting in the conversion of testosterone to estradiol-17β, the latter steroid being necessary for oocyte growth.

Molecular analyses of steroid enzymes from fish are now underway. Genes or cDNAs have been cloned for trout *P*450scc (Takahashi et al., 1992), trout 3β-hydroxysteroid dehydrogenase (Sakai et al., 1994), trout and dogfish *P*450c17 (Tanaka et al., 1991a; Trant, 1995), eel 11β-hydroxylase (Jiang et al., 1996), trout 20β-hydroxysteroid dehydrogenase (Guan et al., 1999), and aromatase from trout (Tanaka et al., 1991b), tilapia (Chang et al., 1997), zebrafish (Callard and Tchoudakova, 1997), and medaka (Tanaka et al., 1995; Fukada et al., 1996; Kobayashi et al., 1996b). Multiple aromatase genes have been identified in goldfish and zebrafish (Callard and Tchoudakova, 1997), and sequence comparisons have revealed that the forms of aromatase expressed in brain and ovary are distinct *P*450 enzymes in goldfish, with approximately 62% similarity at the protein level (Tchoudakova and Callard, 1998). Although it is not yet known whether the catalytic functions of these enzymes differ in a significant way, the brain form of aromatase is expressed at high levels in neural tissue and low levels in ovary, whereas the ovarian form appears to be expressed only in ovary (Tchoudakova and Callard, 1998), suggesting important differences in sex steroid production that may influence the regulation of sex differentiation. Recently, the brain form of aromatase has been shown to be expressed at two distinct levels among individual zebrafish embryos around the time of sex determination (Trant et al., 2001), suggesting a possible role for of this gene in controlling sex differentiation, or that it responds differentially to gonadal development occurring in males and females.

4.2. Cell types involved in sex steroid production

The differentiation of somatic cells in testis and ovary is both morphological and functional. The major roles of these somatic cells are to nourish developing germ tissue and to synthesize and provide the correct hormonal milieu to support either oocyte or spermatocyte development. In testis, interstitial cells analogous to Leydig cells are the major site of androgen synthesis, whereas in ovaries, the two somatic cell layers of the follicle appear to play separate roles in steroid biosynthesis (Hoar and Nagahama, 1978; Nagahama et al., 1982). The ovarian thecal layer contains all enzymes necessary for the production of testosterone and other precursor androgens, whereas the granulosa layer does not synthesize steroids de novo, but is capable of converting testosterone to estrogen via the enzyme aromatase (Nagahama, 1997). Thus, synthesis of estradiol is a two-cell

process (involving both thecal and granulosa cells), a factor which is important for the control of sex-steroid production during sexual maturation (see Section 4.6) (Nagahama and Yamashita, 1988). Some exceptions to this organization have been noted: In common carp, interstitial cells of the ovary also seem to be the main source of androgen production (Epler et al., 1997), and in *Fundulus hetroclitus*, granulosa cells have been shown to synthesize testosterone (Petrino et al., 1989).

Immunohistochemistry has revealed that steroidal enzymes *P*450scc, *P*450c17, 11β-hydroxylase, and 3β-hydroxysteroid dehydrogenase (see Section 4.1) are found in Leydig cells, but not in germ or Sertoli cells, of mature and immature trout testes (Kobayashi et al., 1998; Morrey et al., 1998; Nagahama, 1999). These results strongly indicate that Leydig cells are the major site of steroid synthesis in fish testis. Although aromatase is not usually detectable in testicular tissue, evidence does exist for estradiol production during some stages of testicular development (Pasmanik and Callard, 1988a), suggesting that estrogen synthesis is required in both sexes and that quantitative rather than qualitative differences in sex steroid profiles may be most critical for sex determination. Aromatase activity has been localized in steroid producing cells of the immature ovary by in situ hybridization and immunohistochemistry (Fig. 5).

Sex-steroid production in the gonad seems to require both viable germ cells and functional somatic cells. Thus, in triploid females (which have disrupted germ cells), reduced sex-steroid production and improper development of somatic tissues occurs (Piferrer et al., 1994a; Hussain et al., 1996), whereas in triploid males, gametogenesis still occurs and sex steroid production is normal (Kobayashi et al., 1993b). These observations imply that two-way communication between germ cells and somatic tissues is required for normal steroidogenesis and gonadal development.

Evidence also exists for the local production or conversion of sex steroids in brain cells, implying an important role in the control of gonadal development (Callard et al., 1982). The enzyme aromatase, which is responsible for estradiol synthesis, has been identified in brain (primarily in preoptic–anterior hypothalamic areas) and pituitary tissues (Pasmanik and Callard, 1988a), primarily in gonadotrophs (Melamed et al., 1999). Brain aromatase activity is inducible by in vivo treatment with testosterone and estradiol, and is correlated with seasonal changes in gonadal activity and growth in goldfish (Pasmanik and Callard, 1988a; Pasmanik et al., 1988), suggesting that this enzyme may mediate feedback control of gonadally produced sex steroids to brain (see Section 4.7). Indeed, conversion of androgens (androstenedione) to estrone and estradiol has been observed in *Gasterosteus aculeatus* brains, particularly in the hypothalamus, consistent with a role in controlling the secretion of gonadotropin (Borg et al., 1987). Induction of aromatase activity in the hypothalamus does not occur with a nonaromatizable androgen (e.g. 17α, methyl-dihydrotestosterone) (Pasmanik et al., 1988), suggesting that aromatase control is not directly affected by androgens but is mediated by a positive-feedback mechanism involving gonadal estrogen either directly or indirectly by conversion from testosterone. Although the development of the testis does not require nongonadal estrogen (Miura et al., 1996), the presence of aromatase activity and estrogen production in testis (Pasmanik and Callard, 1988a) may be utilized in males to mediate feedback interactions between the gonad and brain. Consistent with a functional role for brain estrogens, estradiol has also been found to concentrate in the brain in oyster toadfish, *Opsanus tau* (Fine et al., 1990),

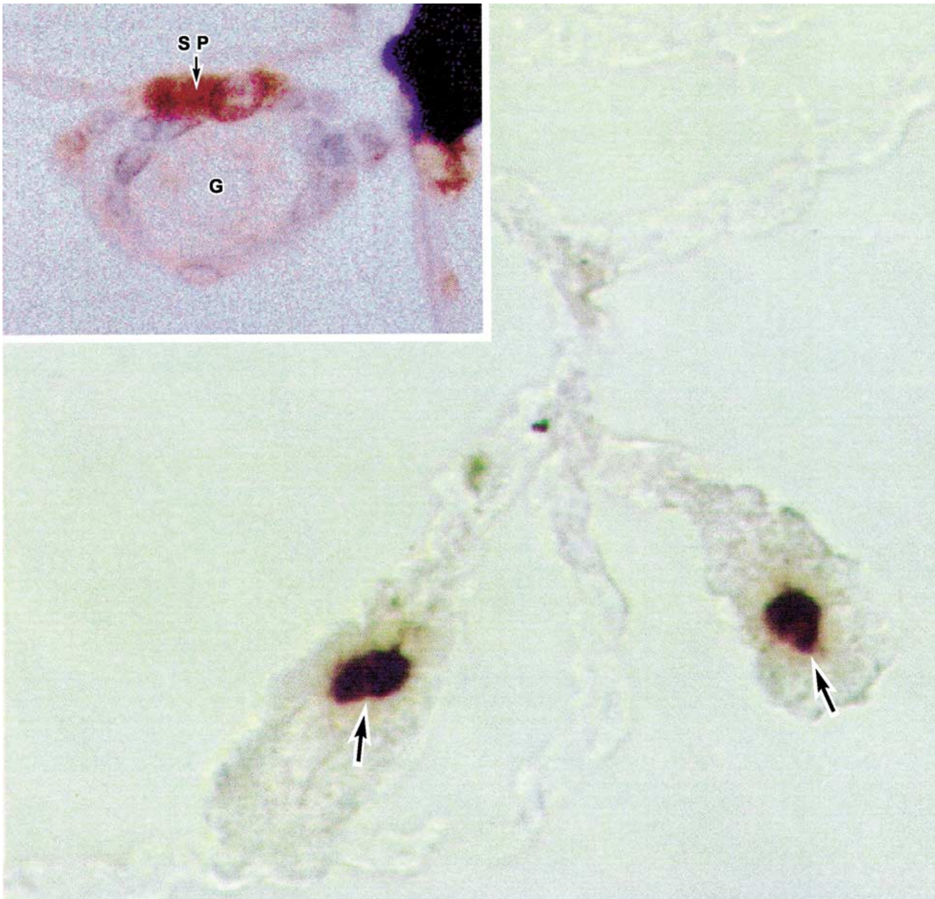


Fig. 5. In situ and protein (inset) localization of aromatase mRNA in gonad of *Oreochromis niloticus* XX female 10 days posthatching. Expression is female-specific and is limited to steroid-producing cells (arrows). G, germ cell. (Unpublished, T. Kobayashi).

and receptors for estrogens have also been identified in fish brain tissue (Pakdel et al., 1990; Begay et al., 1994).

4.3. Ontogenesis of steroid production in fish

It is currently unknown where sex-determination mechanisms intervene in gonadal developmental pathways to initiate alternate courses of steroid production and differentiation. In mammalian systems, the absence of activity from the *Sry* gene on the Y chromosome causes gonadal differentiation to proceed towards a female mode of development, and presence of *Sry* function in gonadal somatic cells (Sertoli cell precursors) induces testicular development (Koopman et al., 1991; Capel, 1998; Koopman, 1999). The former result suggests that biochemical pathways leading to female

development can occur independent of specific actions from the sex-determination gene, and will occur as the default condition in the absence of "interfering" gene activities (see Section 5).

The developmental pathway leading to steroid production in gonadal somatic cells undoubtedly requires complex regulation of a multitude of genes (Swain and Lovell-Badge, 1997) involved in the initial differentiation of steroid-producing cells. It is likely that sex-determination mechanisms do not initiate the process of sex-cell differentiation by acting on totipotent cells, but rather interact at intermediate steps to direct alternate differentiation of gonadal cells and steroid production. Since intracellular gene regulatory events are required to allow differential production of steroids, it is unlikely that the steroids themselves or associated enzymes are the primary and initial factors involved in the determination of sex (see Yamamoto, 1969). Indeed, for medaka, the use of aromatase inhibitors and antiestrogens has revealed that ovary development may occur independent of the action of estrogen (Kawahara and Yamashita, 2000). Nevertheless, steroid production is very closely correlated with very early steps of gonadal differentiation, and can influence long-term decisions regarding sex determination (see Section 6.1). Similarly, if steroid-synthesizing capability is interfered with, sex determination can be disrupted: For example, inhibition of estradiol synthesis in early development using inhibitors of the enzyme aromatase can cause masculinization of coho salmon, rainbow trout, Japanese flounder, and tilapia (Piferrer et al., 1994c; Guiguen et al., 1999; Almeida-Toledo et al., 2000a; Kitano et al., 2000; Afonso et al., 2001).

If steroids are critical for directing initial sex differentiation rather than being a consequence of it, then the appearance of steroid-producing cells and differences in steroid production between the sexes should be apparent prior to morphological differentiation of the gonad (Nakamura et al., 1998). In early development of *Xiphophorus*, an enzyme (3β-hydroxysteroid dehydrogenase) responsible for isomerization and dehydrogenation of steroid precursors has been detected in primitive male and female gonads (Schreibman et al., 1982), indicating that steroid production is probably occurring very early in gonadal differentiation. In *Odontesthes bonariensis*, differentiation of somatic and germinal cells appears to occur simultaneously in both gonad types (Strüssmann et al., 1996a), although testicular development is delayed relative to that of the ovary. Similarly, differentiation of steroid-producing cells in the ovary of amago salmon (Nakamura and Nagahama, 1993) and tilapia (Nakamura and Nagahama, 1985) occurred concomitantly with the onset of stromal aggregation, formation of the ovarian cavity, and meiotic activity of oocytes. Immunohistochemistry has shown that steroidal enzymes *P*450scc, *P*450c17, and 3β-hydroxysteroid dehydrogenase are found at high levels in female gonadal anlagen of tilapia at 10 days posthatch, but was only seen weakly in males and not until 30 days posthatching. Further, the enzyme aromatase was only detected in ovaries (Kobayashi et al., 1998; Nagahama, 1999). In mice, the presence of mRNAs for steroidogenic enzymes including aromatase are present in the fetal gonad before any histological evidence of sex differentiation is apparent (Greco and Payne, 1994). In zebrafish, aromatase mRNA is detectable early in development and levels of the brain form is distributed bimodally among individuals (see above), suggesting an important role for this gene in sex differentiation (Trant et al., 2001).

Other examples indicate that germ and somatic cells may not differentiate synchronously: In medaka, germ-cell differentiation appears to occur prior to that of steroid-producing cells (Satoh, 1974), whereas in sterlet *Acipenser ruthinus*, secretory cells found in the indifferent gonad have steroid-synthesizing structure (Fedorov et al., 1990). In poeciliid species, differentiation of somatic tissues is apparent prior to detectable germ cell differentiation, allowing very early ability to distinguish testis and ovary (Kramer and Kallman, 1985). Since sex-steroid production could be occurring at low levels in the gonad prior to full cytological differentiation of steroid-producing cells, it is difficult to exactly determine the chronology of these events from histological investigations. However, these observations clearly indicate that if steroid production is not responsible for directing early sex differentiation, then the two events are otherwise very closely coordinated.

Testosterone and estradiol levels are normally quite high in fish eggs, arising maternally from the significant levels of sex steroids present in the maturing female (Rothbard et al., 1987; Feist et al., 1990). These egg steroid levels drop during early development, such that by the time sex-determination events are initiated, steroids are at low or undetectable levels that would not override sex determination influences intrinsic to the developing individual. Following early embryogenesis, sex steroids begin to be synthesized endogenously: In tilapia, a bimodal distribution of testosterone levels is observed among individuals at a time (7 weeks postfertilization) when clear histological evidence indicates that sex determination has occurred (i.e. gonadal development has proceeded towards ovarian differentiation in females, or has remained relatively undifferentiated in males) (Rothbard et al., 1987). This bimodality was not detected with estradiol levels, but the levels of testosterone in all-male populations were similar to those found in the low mode of the bisexual population, indicating that female gonads are producing more testosterone than male gonads at 7 weeks. Importantly, these results also demonstrate that the presence of testosterone at higher levels than estradiol does not necessarily result in male development, consistent with testosterone being a very weak androgen in teleosts (Piferrer et al., 1993). The mRNA for 11β-hydroxylase in rainbow trout is found at approximately 100-fold higher levels in testis than ovaries, and in early development is detectable in male gonads prior to testicular differentiation (Liu et al., 2000). The lack of expression of 11β-hydroxylase in females at this time indicates an important role for this enzyme and 11-ketotestosterone in early sex differentiation events in trout.

In salmonids, no significant bimodality of sex-steroid profiles was observed among individual coho salmon larvae (Feist et al., 1990). However, production of testosterone from larval gonadal-tissue explants was higher from all-male than all-female rainbow trout populations (Fitzpatrick et al., 1993). These differences were apparent 2 weeks before the initiation of feeding, at a time prior to significant histological differentiation of the gonads. Estradiol levels could not be detected until much later, but ultimately were higher in the all-female groups. Fluctuating levels and correlations between glucuronide-conjugated and free steroids have also been observed in early trout development (Yeoh et al., 1996), and 17β, hydroxysteroid dehydrogenase activity (see Section 4.1) detected in early arctic char embryos indicates that steroid metabolism could also be playing an important role modulating hormone levels during early sex determination and differentiation (Khan et al., 1997).

4.4. Receptor-mediated action of sex steroids

Steroid action is mediated by binding to specific receptors. The estrogen receptor can be found in a variety of gonadal and other tissues, including liver, ovary, heart, spleen, muscle, and brain (Smith and Thomas, 1990). Estrogen receptor levels are themselves influenced by estrogens (Mommsen and Lazier, 1986), mediated by effects on estrogen receptor gene expression (MacKay et al., 1996; Pakdel et al., 1997). Such positive control of estrogen receptors by estrogen ensures that sufficient receptor is available in stimulated cell types to respond to the full degree of estrogen presentation.

Estrogen receptors are DNA-binding proteins that influence gene expression (e.g. *Vitellogenin*, see Section 4.5) (Lazier et al., 1985), but may also function via cell-surface pathways (Kelly et al., 1999). Different genes have distinct thresholds of estrogen concentrations they will respond to: for example in cultured trout hepatocytes, the estrogen receptor gene is induced at a lower level of estradiol-17β than is the vitellogenin gene (Flouriot et al., 1996). Estrogen receptor concentrations also fluctuate in different tissues, depending on the physiological state of the animal: For example, livers from previtellogenic females have very much lower levels of estrogen receptor than vitellogenic individuals (Smith and Thomas, 1990). Estrogen receptor mRNA has also been detected in the pituitary and hypothalamus in trout (Pakdel et al., 1990), which may mediate steroidal control of GnRH and GtH secretion (see Section 4.7). Similarly, estrogen receptors are also found in the pineal gland (Begay et al., 1994), and since estrogens have been shown to affect the release of melatonin, endocrine-disrupting xenobiotics could potentially influence seasonal control of spawning by this mechanism. Recently, a second form of estrogen receptor has been cloned from tilapia (Chang et al., 1999b), catfish (Xia et al., 1999) and goldfish (Tchoudakova et al., 1999), indicating that fish possess two estrogen receptors typical of the α and β forms found in higher vertebrates. In tilapia, while both genes are expressed in early male and female gonads at 10–15 days posthatch (Nagahama, 1999), they display different expression patterns suggesting important roles in regulating early sex differentiation.

Proteins capable of binding androgens have been identified in testicular tissues of fish (Foucher and Le Gac, 1989). Androgen receptors are found in gonadal tissues in both Agnatha and Osteichthyes, suggesting that this protein has played an important role in sex steroid function throughout the evolution of the fishes (Fitzpatrick et al., 1995). Androgen receptors have also been found in the cytosol of ovaries (Fitzpatrick et al., 1994), providing a plausible mechanism by which androgen-mediated sex reversal could be occurring in female gonads of fish (see Section 6.1). Similarly, the presence of androgen receptors in ovarian tissues provides a direct pathway by which environmental androgens could cause reproductive impairment. Recently, multiple forms of androgen receptors have been cloned from trout and eel (Ikeuchi et al., 1999; Takeo and Yamashita, 1999; Todo et al., 1999).

4.5. Hormonal control of vitellogenesis

Estradiol acts at the level of the gonad to stimulate oocyte development, but also acts indirectly by controlling the expression of genes in nongonadal tissues that are necessary

for oocyte growth. For example, major proteins induced by estradiol in liver include Choriogenin H required for the formation of egg chorion (Murata et al., 1997), and vitellogenin, a phospholipoprotein complex required for oocyte growth and used for larval energy supplies. These proteins are primarily produced from liver cells in females, and are transported to the ovary via blood. Being produced at much higher levels in females than males, vitellogenin can provide a convenient assay (Le Bail and Breton, 1981; Gordon et al., 1984; Norberg and Haux, 1988; Kwon et al., 1990; Matsubara and Sawano, 1992; Kanamori et al., 1993; Perez and Callard, 1993; Miura et al., 1994; Heppel et al., 1995a,b; Mourot and Le Bail, 1995; Tyler et al., 1996; Bon et al., 1997; Takemura and Oka, 1998) for determining phenotypic sex of fish or for evaluating potential feminizing effects arising from exposure to pollution (see also Section 6.4).

Estrogens have been shown to stimulate vitellogenin protein or mRNA in several teleost species, both in vivo and in in vitro cultures of hepatocytes (Maitre et al., 1985; Olin and Von Der Decken, 1987; Le Guellec et al., 1988; Kwon et al., 1993; Kim et al., 1997b). A small amount of vitellogenin synthesis can also be detected in testis in response to estrogen treatment in *Oreochromis aureus* (Ding et al., 1993). The estrogen receptor blocking agent tamoxifen can reduce hepatic vitellogenin mRNA levels, despite increased circulating estradiol levels arising from blockage of feedback inhibition of steroid synthesis (Peyon et al., 1997). This finding is consistent with vitellogenin gene expression occurring in response to binding of estrogen receptors with estrogen-response elements associated with regulatory regions of the vitellogenin gene promoter. Vitellogenin gene expression is stimulated over a wide range of estrogen concentrations, much above that needed to induce maximal levels of estrogen receptor gene expression (Lazier et al., 1996). A number of xenoestrogens that mimic the action of estrogens have also been shown to induce vitellogenin production (Sumpter and Jobling, 1995), forming the basis of screening assays for effects of environmental pollutants on reproduction in fish (see Section 6.4).

Androgens have also been shown to induce vitellogenin production in cultured trout and eel hepatocytes (Peyon et al., 1997; Mori et al., 1998), and this effect is blocked with tamoxifen, indicating stimulation is occurring through the estrogen receptor. Interestingly, the in vivo effects of androgens are different: vitellogenin synthesis was not affected by androgens in vivo in catfish (Sundararaj and Nath, 1981) or eels (Peyon et al., 1997), whereas treatments of tilapia with 17α-methyltestosterone does dramatically reduce circulating vitellogenin protein and hepatic vitellogenin mRNA levels (Lazier et al., 1996). In this latter case, androgen treatments also lowered serum estradiol levels in females, suggesting that effects may be occurring by feedback of steroids at the hypothalamic or hypophyseal levels.

Vitellogenin appears to be involved in feedback control of estrogen levels in rainbow trout. Treatment of females with vitellogenin reversibly reduces circulating estradiol levels, and oocytes treated in vitro with vitellogenin show decreases in estradiol synthesis (Reis-Henriques et al., 1997). These observations indicate that estradiol and vitellogenin co-regulate each other via a negative feedback control loop. Vitellogenin synthesis by hepatocytes can also be directly influenced by growth hormone and prolactin in vitro in fish (Kwon, 1997), and the reciprocal stimulation of GH and estradiol (Zou et al., 1997) suggest that somatotropins (and IGFs, see above) may also play a role in controlling vitellogenin production in vivo by modifying steroid levels.

4.6. Hormonal control of sexual maturation

In fish, sexual maturation is central to sex differentiation since it signals the end of the growth phase of gonad development. In some species, individuals die following sexual maturation and spawning, whereas in other fish a subsequent wave of germ-cell differentiation and growth (recrudescence) occurs for future spawning periods.

During sexual maturation, dramatic shifts in steroid biosynthetic activity occur in both sexes, having effects on both somatic (e.g. Lebail, 1981; Beacham and Murray, 1983, 1986; Kramer et al., 1988) and gonadal tissues. In females, the production of testosterone and estradiol by the ovary are usually reduced, and production of 17α, 20β, dihydroxy-4-pregnen-3-one (17α, 20β-DP) is dramatically enhanced (Goetz, 1983; Sakai et al., 1987; Nagahama, 1994, 1997). It is the rise in 17α, 20β-DP rather than a decline in other steroids during the final phases of sexual maturation that is responsible for inducing final maturation of oocytes (Kobayashi et al., 1987). However, 17α, 20β DP production itself may be a consequence of reduced estradiol synthesis since the aromatase inhibitor Fadrozole is capable of prematurely inducing 17α, 20β DP production in preovulatory coho salmon Afonso et al., 1999a,b). An enzyme (20β hydroxysteroid dehydrogenase) responsible for conversion of 17α hydroxyprogesterone to 17α, 20β DP has been recently characterized (Guan et al., 1999), and this enzyme has been shown to be induced in granulosa cells by gonadotropin. Production of 17α, 20β DP during sexual maturation has now been observed in many fish species (Young et al., 1982, 1983c; Goetz, 1983; Nagahama et al., 1983; Ueda et al., 1983; Scott et al., 1984; Van der Kraak et al., 1984, 1985; Levavi Zermonsky and Yaron, 1986; Goetz et al., 1987; Nagahama, 1987; Kobayashi et al., 1988; Mayer et al., 1992; Frantzen et al., 1997; Mylonas et al., 1997a; Estay et al., 1998). In multiple spawners, and some single spawners, levels of 17α, 20β DP still cycle with sexual maturity, but estradiol levels can remain relatively high through maturation, and continued recruitment of oogonial cells occurs throughout the spawning period to allow ovary development for the next cycle (Levavi Zermonsky and Yaron, 1986; Drori et al., 1994; Rinchard et al., 1997). The mechanism by which 17α, 20β DP causes egg maturation involves a complex interaction between oocyte 17α, 20β-DP receptors, prostaglandins PGE1, PGE2, PGF1α, PGF2α (Kobayashi and Stacey, 1993), and signal transduction pathways operating via inhibitory G proteins (Yoshikuni and Nagahama, 1994; Goetz and Garczynski, 1997; Nagahama, 1999). The intracellular events inducing maturation involve the derepression of cyclin-B mRNA translation by polyadenylation (Katsu et al., 1997), allowing production of this protein, which in turn complexes with egg stores of cdc-2 kinase (Yamashita et al., 1995; Nagahama, 1997). The cyclin B and cdc-2 complex are phosphorylated to form maturation promoting factor (MPF), which in turn induces germinal vesicle breakdown (GVBD) and oocyte maturation.

Other steroids (e.g. 17α, 20 β, 21-trihydroxy-4-pregnen-3-one) have also been shown to synthesized by gonadal tissues (Canario and Scott, 1989; Trant and Thomas, 1989), interact with specific gonadal receptors (Patino and Thomas, 1990; Pinter and Thomas, 1995), and are capable of inducing oocyte maturation (Trant and Thomas, 1988; Canario and Scott, 1990), indicating that a variety of steroids may act as maturation hormones among different fish species.

Changes in steroid biosynthesis during maturation in females is mediated in part by a reduction in the amount of ovarian aromatase enzyme, and consequently reduced conversion of testosterone to estradiol in the follicle (Young et al., 1983a). This control is exerted at the mRNA level, probably by affecting the transcription rate of the aromatase gene (Trant et al., 1997), which may be mediated by the transcription factor FTZ-F1 (Tanaka et al., 1995; Watanabe et al., 1999). However, alterations in steroid synthesis also occur at higher levels in the steroid synthetic pathway. At the onset of maturation, 17α-hydroxyprogesterone conversion to androstenedione and testosterone is dramatically reduced, and the pathway leading to 17α, 20β-DP is enhanced (Kobayashi et al., 1996a). These shifts in steroid profiles imply that a reduction in 17β-hydroxysteroid dehydrogenase and C17, 20 lyase activities are probably responsible (Sakai et al., 1988). How this latter enzyme activity is reduced is currently unclear: The production of 17α-hydroxyprogesterone (the precursor to 17α, 20β-DP) requires the activity of 17α-hydroxylase, but genetic studies have revealed that this enzyme activity (needed for pregnenolone to 17α-hydroxypregnenolone conversion) and C17, 20 lyase activities are found within the same protein in two classes (dogfish and trout) of fish (Lin et al., 1993; Trant, 1995, 1996). Thus, blockage of androstenedione synthesis may be occurring by the selective inhibition of C17, 20 lyase activity within this protein, or by the suppression of *P*450c17 production and activation of another gene encoding only 17α-hydroxylase activity. Within the *P*450c17 enzyme, the C17, 20 lyase activity requires higher levels of *P*450 reductase for maximal activity than does the 17α-hydroxylase activity, which may allow independent regulation of these activities (Lin et al., 1993). Calcium-mediated signaling may also play a role, since C17, 20 lyase activity can be enhanced by inhibition of phosphodiesterase activity, an effect that can be blocked with a calcium ionophore or by activating protein kinase C (Kobayashi et al., 1996a). Barry et al. (1990) hypothesized that during the high level of steroid synthesis occurring during the gonadotropin surge at final maturation, C17, 20-lyase may become saturated with 17α-hydroxyprogesterone and the excess diffuses out of testicular somatic cells to spermatozoa where it is metabolized to 17α, 20β-DP. The latter hormone may inhibit C17, 20 lyase activity to cause a positive feedback circuit, which results in a very rapid switch from androgen to 17α, 20β-DP synthesis (Barry et al., 1990).

The redirection of steroid synthesis makes 17α-hydroxyprogesterone (produced in Leydig cells and thecal cells) available for conversion to the maturation hormone 17α, 20β-DP (Nagahama and Yamashita, 1988), a reaction which is catalyzed by 20β-hydroxysteroid dehydrogenase. This enzyme is found in spermatozoa and other germ cells in the testis (Hourigan et al., 1991; Asahina et al., 1993; Vizziano et al., 1996b), and granulosa cells of ovarian follicles (Nagahama and Yamashita, 1988). This enzyme is not restricted to maturing gonads, but can also be found in immature trout testicular tissues that can produce 17α, 20β-DP in response to type-II gonadotropin (LH) (Vizziano et al., 1995). Activity of 20β-hydroxysteroid dehydrogenase can also be found in extragonadal tissues in salmon, trout, and goldfish, suggesting that significant production of 17α, 20β-DP may occur in other tissues (Sangalang and Freeman, 1988; Ebrahimi and Kime, 1998).

In males, androgen production remains high throughout sexual maturation, even during the period of 17α, 20β-DP synthesis (Fitzpatrick, 1985; Mayer et al., 1990, 1992; Schulz

et al., 1994). In vitro, testis utilize progesterone more effectively than pregnenolone, whereas ovaries can utilize both precursors equally effectively (Sangalang and Freeman, 1988), indicating that the steroid pathways leading to 17α, 20β-DP differ between the sexes. In rainbow trout, in vitro studies have shown that only low levels of estradiol synthesis occur in male trout, but these are reduced even further at the onset of maturation. Estradiol treatment can inhibit production of 17α, 20β-DP production in vitro in testis (Vizziano et al., 1996a), indicating that reproductive impairments arising from environmental estrogenic compounds could be in part due to disruption of testicular 17α, 20β-DP production, which could in turn affect the actions of this hormone in both maturation and pheromonal stimulation of reproductive behaviours (e.g. Van der Kraak et al., 1989; Sorensen and Goetz, 1993).

4.7. Neuroendocrine control of gonad development

Gonadal development in both sexes is dependent on endocrine communication between the brain, pituitary and gonads, allowing developmental, physical, chemical, social and seasonal cues to be integrated with gonad maturation (Bieniarz and Epler, 1992; Yaron, 1995; Trudeau, 1997). A primary source of control for this pituitary–gonadal axis is mediated through the production of gonadotropins (GtH) in the pituitary gland, of which there are two main types (Suzuki et al., 1988b; Swanson et al., 1991; Van der Kraak et al., 1992a). Prior to maturation, GtHI (a gonadotropin analogous to mammalian follicle stimulating hormone, FSH) is produced that promotes steroid synthesis and gonadal growth and differentiation throughout much of a fish's life (Miura et al., 1991; Van Winkoop et al., 1994; Miura et al., 1996; Nagahama, 1999). This process has been well elaborated for the eel testis that can complete development of all stages of spermatogenesis in vitro in defined culture conditions (Miura et al., 1996). In this case, the interaction of gonadotropin with receptors on Leydig cells suppresses the production of proteins (ZP2 and ZP3), which may inhibit spermatogenesis (Miura et al., 1998), and induces 11β-hydoxylase and 11β-hydroxysteroid dehydrogenase enzyme activities and hence 11-ketotestosterone production. This latter hormone induces Sertoli cells to synthesize activin β_B, which is capable of inducing premeiotic spermatogonial proliferation (Nagahama et al., 1997). Activin β_B and its receptor cDNAs have recently been cloned from goldfish ovary, and activin β_B is found to be expressed both in gonadal and nongonadal tissues, suggesting a role wider than control of gametogenesis (Ge et al., 1997a,b).

During the period of sex determination in the gonochoristic rainbow trout, GtHI first appears in the larval pituitary gland at a time when gonadal sex cells begin to divide (Saga et al., 1993) but no differences in distribution of GtH-I reactive cells are observed between males and females (Feist and Schreck, 1996). Similarly, in *O. bonariensis*, both GtH1 and GtH2 protein are immunohistochemically detectable in larval pituitary glands just prior to and during the sex determination period (Miranda et al., 2001). Although larval GtH synthesis may be stimulated by steroid production from the newly differentiating gonad, it is also possible that gonadotropin is important for stimulating the development of the early indifferent gonad (Van Winkoop et al., 1994). Gonadotropins have been shown to play a critical role in sex differentiation in hermaphroditic species (see Section 4.9). As sexual maturation approaches, a switch occurs in the pituitary gland such that a new form of

gonadotropin is produced (GtHII), which is analogous to mammalian leutenizing hormone (LH). The production of LH induces receptor-mediated changes in gonadal enzymes and steroid production [e.g. 11β-hydroxylase (Jiang et al., 1996), and aromatase (Kagawa et al., 1982; Young et al., 1983a)], which results in production of 17α, 20β-DP and induced germ-cell maturation as described in Section 4.6.

The production and release of different forms of gonadotropins (determined by the expression of different β subunits which combine with a common α subunit) from the pituitary gland is influenced by sex steroids and gonadotropin-releasing hormones (GnRHs) involving a Ca^{+}-dependent process (Levavi-Sivan and Yaron, 1993; Bhattacharya et al., 1994; Melamed et al., 1998, 2000). Secreted gonadotropins interact with the gonad by binding to specific receptors (see below), which influence gene expression in recipient cells via second messenger processes involving cAMP (Young et al., 1983b; Kanamori and Nagahama, 1988). Administration of gonadotropins, or gonadotropin-release hormones such as LHRH, can accelerate the onset, and increases the magnitude, of 17α, 20β-DP production, and thereby promote ovulation or spermiation. For example, injection of preovulatory chinook salmon females with pituitary GtH extracts (or gonadotropin-releasing hormones, which in turn affect GtHs) around the time of maturation reduces circulating estradiol levels, increases 17α, 20β-DP, and can induce early ovulation (Van der Kraak et al., 1983, 1985). Similarly, in males, LHRH or GtH treatment increases testosterone levels and/or spermiation in carp (Ngamvongchon et al., 1987) and trout (Schulz et al., 1992). Similar effects have been observed in many other studies and other fish species (e.g. Hirose et al., 1983; Van der Kraak et al., 1984, 1985; Fitzpatrick et al., 1987; Zhao and Wright, 1988; Zhao et al., 1988; Schulz and Bluem, 1990; Nagahama, 1994; Mylonas et al., 1997b,c). Evidence has also been presented that growth hormone can accentuate the steroid-enhancing effects of gonadotropin in the goldfish ovary, although this somatotropin seems not to have an effect in isolation (Van der Kraak et al., 1990).

In some fish species, GtH release from the pituitary is under negative control by dopamine (Lin and Peter, 1996). Treatment of male goldfish with dopamine (or a dopamine agonist, apomorphine) suppresses GtH levels (Chang and Peter, 1983a), and, conversely, dopamine antagonists such as domperidone are able to enhance the actions of GnRH and increase GtH levels (Lin and Peter, 1996). Dopamine appears to act (in part) downstream of neural inhibition of GtH release since fish with high GtH levels arising from preoptic ablations still show reductions in GtH with dopamine or apomorphine treatment (Chang and Peter, 1983a). Treatment of tilapia pituitary fragments or cells with cAMP, Ca^{2+} ionophores, or inhibitors and agonists involved in receptor signal transduction reveal that dopamine inhibition acts downstream of protein kinase C and Ca^{2+} influx, and upstream of arachidonic acid (Chang et al., 1993; Levavi-Sivan et al., 1995). Nicotine also controls GtHII production by causing direct release from pituitary cells in common carp (Mikolajczyk et al., 1993), indicating potential cholinergic regulation of secretion, but this effect seems to be inhibited through dopaminergic action in vivo (Mikolajczyk et al., 1998). Other factors involved in regulating GtH secretion include neuropeptide-Y (NPY) (Peng et al., 1993), bombesin (Himick and Peter, 1995), serotonin (Somoza and Peter, 1991), and GABA (Trudeau et al., 1993a), which act by accentuating GnRH production. These latter effectors may also be influenced by seasonal changes and sex steroids levels (Trudeau, 1997).

Once released from the pituitary, gonadotropins circulate via the bloodstream and interact with receptors on target tissues. GtHI and GtHII binding studies have indicated the presence of two gonadotropin receptors in the fish gonad: One receptor (GtHRI) binds both GtHI and GtHII, whereas another (GtHRII) binds only GtHII (Miwa et al., 1994). Recently, a GtH receptor has been cloned and characterized from amago salmon ovarian follicles; both GtHI and GtHII interacted well with this receptor and elevated cyclic AMP levels in transfected COS cells expressing the receptor clone (Oba et al., 1999). In the vitellogenic ovary, GtHI receptors are found on both thecal and granulosa cells (intensely on the latter), whereas in the preovulatory follicle, they are only found on thecal cells. These results are consistent with the stimulatory effects that gonadotropins (and indirectly their releasing hormones) have on steroid biosynthesis in vitellogenic ovaries (Kagawa et al., 1984; Zhao and Wright, 1986; Haddy and Pankhurst, 1998), and suggest that the switch in steroid synthesis that occurs at maturation arises in part through GtHI receptor-controlled destimulation of granulosa cells. Similarly, GtHII receptors are not found in vitellogenic ovaries, but are found on granulosa cells of preovulatory follicles (Miwa et al., 1994). Thus, in granulosa cells, aromatase activity and estradiol production are correlated with GtHI interaction with its receptor, whereas 20β-hydroxysteroid dehydrogenase mRNA and enzyme activity (required for 17α, 20β-DP synthesis) are induced in granulosa cells in response to GtHII binding (Nagahama, 1997). The expression of GtHII receptors (followed by ligand activation) in granulosa cells may inhibit the production of GtHI receptors. Such a control would result in blockage of aromatase and estradiol synthesis, and would make precursor steroids from thecal cells available for the production of 17α, 20β-DP as described above.

In males, GtHI receptors are found at all stages of spermatogenesis, but GtHII receptors are only detected on Leydig cells during the time of spermiation (Miwa et al., 1994). These findings are consistent with GtHII being responsible for allowing the 17α, 20β-DP precursor 17α-hydroxyprogesterone to accumulate in these cells during maturation. In vitro treatment of testis with gonadotropins at different stages also revealed that GtHII had a greater capability than GtHI to stimulate 17α, 20β-DP from late stage testis (Planas and Swanson, 1995).

Communication between the brain and the developing gonad is required to ensure appropriate GtH levels and rates of gonadal development and timing of maturation. Gonadotropins are well known to stimulate steroid synthesis in the teleost gonad, and sex steroids are able to feed back to the brain to alter GtH production. Thus, if gonadal tissue is removed from male Atlantic salmon, sex steroid and plasma and pituitary gonadotropin levels decline, and this effect can be ameliorated by androgen replacement (Borg et al., 1998). Similarly, in triploid salmon that normally have low steroid levels, estradiol treatment increases synthesis of pituitary GtH levels (Benfey et al., 1989), and in eels, in vivo androgen and estrogen treatment can induce brain and pituitary GnRH and pituitary GtH levels (Montero et al., 1995). Negative regulation of GtH release by steroids is also indicated since circulating levels of GtH do not necessarily increase with estradiol treatment (Benfey et al., 1989). Similarly, in experiments with unilaterally ovariectomized trout, estrogen levels drop and GtH levels rise in correlation with a (compensatory) recruitment of primary oocytes into vitellogenesis (Tyler et al., 1997). Curiously, in eels, it has been found that androgens but not estrogens stimulate the expression of the

gonadotropin subunit IIβ gene in pituitary explants in vitro (Huang et al., 1997), suggesting that direct and indirect (via aromatization) regulatory influences of sex steroids may be occurring. The exact levels of sex steroids as well as developmental differences in sensitivity to them also appear to play a role since, in immature tilapia pituitary cells, LH subunit β mRNA increases with testosterone treatment, whereas FSH subunit β mRNA is increased by low, but decreased by high, levels of testosterone, and is not increased in regressed males (Melamed et al., 1997, 1998). Androgen receptors have been identified in the goldfish brain (Pasmanik and Callard, 1988b), but the lack of estrogen receptors in GnRH-producing cells in trout (Navas et al., 1995) suggests that feedback control may be mediated by other cell populations in the brain.

In goldfish, treatments with testosterone or estrogen do not elevate GtH levels in vivo (Trudeau et al., 1993b), but these hormones do increase dopamine turnover rates and elevate levels of this neurotransmitter in the brain. Since dopamine has been shown to inhibit GnRH and consequently GtH secretion, elevated dopamine levels are able to counter any stimulatory effect from steroids to maintain GtH at normal levels (Trudeau, 1997). In tilapia, dopamine also inhibits GtH (LH) release from pituitary cells, but it does not affect mRNA levels for this gene, suggesting it inhibits LH secretion rather than production (Melamed et al., 1996). Treatment of fish with tamoxifen (an estrogen receptor blocker) can induce ovulation (Donaldson et al., 1981), perhaps by releasing dopamine inhibition of GnRH synthesis and GtHII release during sexual maturation (Chang and Peter, 1983b).

Neural control of gonadal development may also be mediated by direct innervation. Steroid producing (Leydig) cells are directly innervated in the clingfish (*Sicyases sanguineus*) testis (Perez et al., 1983), and, more recently, thecal cells in tilapia (*O. niloticus*) (Nakamura et al., 1996) have been shown to be innervated.

4.8. Steroidal control of sex differentiation in hermaphrodites

The occurrence of sex-changing fish (see Section 3.2) demonstrates that developmental pathways between testicular and ovarian differentiation can be influenced postzygotically. Investigations of hormone profiles in sex-changing fish have implicated gonadal steroids as well as brain and pituitary hormones in this process. However, the existence of synchronous hermaphrodites (which allow maturation of both male and female germ tissue simultaneously) reveals that exposure to changing levels of circulating hormones probably are only partly involved in the sex differentiation process. Local, paracrine influences and/or differences in reception of hormonal signals probably also play significant roles.

In the protandrous black porgy *A. schlegeli*, sex hormone levels peak just before the spawning season (Chang and Yueh, 1990b), and although testosterone levels are found to be similar between males, females and sex-reversing females, estradiol is higher in the latter two compared to males (Chang et al., 1994). In general, testosterone levels do not provide reliable indications of the state of gonadal differentiation (e.g. Yeung and Chan, 1987c), whereas the presence of 11-oxygenated androgens has been correlated with the testicular phase in protandrous fish. Similarly, a rise in estradiol levels concomitant with the sex-reversal process usually occurs. Estradiol levels in the protandrous *Sparidentex hasta* (Kime et al., 1991), sea bass *L. calcarifer* (Guiguen et al., 1993), and anemonefish

Amphiprion melanopus (Godwin, 1994a) are higher in females than males, and the male-associated androgens 11-ketotestosterone and 11β-hydroxytestosterone were found to be higher in males than females, as is the case for gonochorists. Concordantly, in protandrous *Pagellus acarne* and *L. calcarifer*, the presence of these steroids has been correlated with enzyme activities for 11β-hydroxysteroid dehydrogenase and 11β-hydroxylase in testicular tissues, and aromatase activity in ovary (Reinboth et al., 1986; Guiguen et al., 1995). During the course of sex reversal in *A. melanopus*, all androgen levels examined declined, but estradiol levels did not show a rise until after ovarian development was underway, implying that circulating estradiol may be a consequence of sex reversal rather than a cause (Godwin, 1994a).

In vitro studies with gonadal tissues from another protandrous anemonefish, *A. frenatus*, has shown that different tissue types from the gonad have different steroid synthesizing capabilities, notably 11-ketotestosterone in testicular tissue, and both testosterone and estradiol in ovary (Nakamura et al., 1994). Discrete steroid synthetic capabilities reflective of the gonadal tissue type again suggests the importance of local differentiation and paracrine action of these hormones.

Administration of sex steroids has shown they can play a direct role in sex reversal of protandrous fish. For example, treatment of black sea bream (*Sparus macrocephalus*) with diethylstilbestrol can induce feminization a year prior to the normal time of sex reversal (Ruan et al., 1996), and administration of estradiol to black porgies *A. schlegeli* also enhances sex reversal to females (Chang et al., 1994, 1995b). Estradiol treatment reduces the synthesis of testosterone and 11-ketotestosterone, and increases aromatase enzyme activity (Chang and Lin, 1998) in *A. schlegeli*, but androgen levels rise again after cessation of estradiol treatment, suggesting that endogenous controls take some time to be overridden in this protandrous species. Combined, these results indicate that estradiol plays a direct role in sex reversal in protandrous species by suppressing masculinizing steroids and stimulating enzymes needed for estrogen synthesis.

In protogynous fish, androgen and estrogen levels have also been shown to correlate with sex change. In the stoplight parrotfish, *Sparisoma viride*, sex change is correlated with a dramatic rise in plasma 11-ketotestosterone and a reduction in estradiol (Cardwell and Liley, 1991). Similarly, 11-ketotestosterone (plasma levels or in vitro synthesis rates from gonadal tissues) is generally higher in males, and estradiol is higher in females, in other protogynous species, including groupers *Epinephelus morio* (Johnson et al., 1998) and *E. akaara* (Tanaka et al., 1990a), black sea bass *Centropristis striatus* (Cochran and Grier, 1991), and the wrasses *Pseudolabrus japonicus* (Morita et al., 1997), *Coryphopterus nicholsi* (Kroon and Liley, 2000), and *T. duperrey* (Hourigan et al., 1991). In *T. duperrey*, the enzyme aromatase is expressed in the protogynous ovary until sex change is initiated, at which point expression levels decline to undetectable levels in developing testis (Morrey et al., unpublished in Nagahama, 1999). In the synchronous hermaphrodite *Serranus subligarius*, in vitro incubations of testicular regions of gonads with pregnenolone produce primarily 11-ketotestosterone and 11β-hydroxytestosterone, whereas the dominant product from ovarian tissues comigrates with a novel trihydroxylated progesterone (Oliver, 1991).

Treatment of protogynous hermaphrodites with androgens (either 11-ketotestosterone, or the synthetic androgen 17α-methyltestosterone) can masculinize and enhance sex-

reversal in *S. viride* (Cardwell and Liley, 1991), as well as groupers *Mycteroperca microlepis* (Roberts and Schlieder, 1983), *Epinephelus suilus* (Tan-Fermin et al., 1994) and *E. aeneus* (Hassin et al., 1997). Withdrawal of 17α-methyltestosterone treatment in this latter species results in reversion back to an ovarian condition (Tan-Fermin, 1992), analogous to the effect described above for estrogen withdrawal and transient sex reversal in the protandrous *A. schlegeli.* Treatment of female blackeye gobies, *C. nicholsi*, with androgens or an aromatase inhibitor were both capable of causing sex reversal (Kroon and Liley, 2000), indicating the importance of androgens, and possibly lack of estrogens, on sex differentiation in this species. Gonadal transformation is not always complete with these hormone treatments: In bluehead wrasse (*T. bifasciatum*) implanted with testosterone (Kramer et al., 1988), ovarian degeneration occurred without testicular development, however transformation of the blue head colour (a secondary sex character) was complete.

4.9. Neuroendocrine control of gonadal differentiation

The changes occurring in sex-reversing fish do so in step with behavioural (see Section 6.3) or seasonal cues, and are therefore linked to brain function and regulation. Thus, in vivo or in vitro treatments of protandrous fish with gonadotropin or hormones affecting its release have been found to have a general influence on gonadal steroid production, as occurs in gonochorists (see Section 4). In species with gonads that are hermaphroditic early in development, sex differentiation can also be influenced. For example, treatment of *Anguilla dieffenbachii* with salmon pituitary extracts enhances testicular and ovarian tissues (Lokman and Young, 1998).

Among protandrous species, gonadotropins or their releasing hormones (e.g. LHRH and analogues) have been shown to increase testosterone in males or bisexuals, and testosterone and estradiol in females (Chang et al., 1991; Nakamura et al., 1994). LHRH treatments also increases oocyte growth, as does treatment with an antiestrogen, the latter perhaps acting by release of negative regulation by estrogens at the level of the pituitary gland (Chang and Yueh, 1990a) (see Section 4.7). Similarly, although no effect of LHRH treatment on steroid hormone levels was observed in *S. aurata*, treatments did result in a reduced percentage of testicular tissue in the gonad (Vilia and Canario, 1995), suggesting that gonadotropins are involved in sex transformation in this protandrous species. In vitro treatment of *S. aurata* gonadal tissues with human chorionic gonadotropin (hCG) strongly suppressed radioactive androstenedione to testosterone conversion in testis but not in ovary (Eckstein et al., 1978), indicating a possible mechanism by which sex reversal may be mediated. Gonadotropins and their releasing hormones seem to be differentially regulated between the sexes in *S. aurata* (Elizur et al., 1995), and higher numbers of GnRH-producing cells are observed in the preoptic area of the brain in males than females in *A. melanopus* (Elofsson et al., 1997). These results suggest that fluctuating levels of gonadotropins (and/or ratios of FSH- vs. LH-types) may play a role in sex transformation in protandrous fish.

Treatments with gonadotropin or gonadotropin-releasing hormones are also able to induce sex reversals in protogynous fish, resulting in the production of germ cells (spermatogonia, spermatocytes, and spermatids) and somatic (Leydig) cells. In *Synbranchus marmoratus* (Ravaglia et al., 1997) and *M. albus* (Tao et al., 1993), treatment with a

salmon GnRH analogue elevated androgen levels and induced functional sex reversals, although in the latter species the sex-reversing effects of GnRH or nonpiscine gonadotropins may be more effective in postspawned than prespawned females (Yeung et al., 1993a,c). HCG induces precocious sex reversal in *C. julis* (Reinboth and Bruslé Sicard, 1997), and, in *T. bifasciatum*, hCG injection can rapidly induce the appearance of testicular tissue in females within 6 weeks in most animals (Koulish and Kramer, 1989). Peptides regulating gonadotropin production such as GnRH analogues (Kramer et al., 1993) or NPY (Kramer and Imbriano, 1997) also can strongly influence sex reversal in protogynous species (*T. bifasciatum*). Interestingly, in the same species, enhanced numbers of GnRH-producing cells in the preoptic area of the brain are correlated with sex-transformed individuals (Grober and Bass, 1991). Together, these findings provide strong evidence for the involvement of hypophyseal and pituitary-level controls in mediating environmental cues for sex change in these protogynous species. Evidence has also been presented for the important role of GtH2 and specific forms of GnRH in testicular differentiation in the gonochoristic sea bream, *D. labrax* (Rodriguez et al., 2000).

4.10. Extragonadal metabolism and secretion of sex steroids

Circulating sex steroid levels in fish are maintained by a balance of synthesis, degradation, solubilization, and excretion of steroids (Andersson and Foerlin, 1992). The bioactivity of steroids in plasma can be modulated by their availability in free form, by steroid binding proteins, (Foucher et al., 1991, 1992; Laidley and Thomas, 1997), and/or by conjugation with glucuronic or sulfuric acid (Schulz, 1986; Yeoh et al., 1996). Whereas much (but not all) steroid synthesis occurs in the gonad, the major site of catabolism of steroids and steroid conjugates is in the liver. The enzymes involved in extragonadal metabolism of steroids belong principally to the cytochrome *P*450 family, which is a large and diverse group of proteins involved in oxygenation of xenobiotics, steroids, and other compounds (Stegeman, 1993). Such enzymes have the capability to alter the hormonal milieu of a developing embryo, and thus potentially also sex differentiation.

When labeled testosterone is injected intra-arterially into rainbow trout or flounder, the metabolites isolated in bile are primarily forms of dihydrotestosterone, and all are found to be glucuronide derivatives (Truscott, 1983). Thus, efficient glucuronide conjugation and hepatic steroid 5α-reductase activities appear to be involved in androgen metabolism in fish. Androgen metabolism associated with the enzymes 17-hydroxysteroid dehydrogenase, 6β-hydroxylase, and 16β-hydroxylase enzyme activities have also been identified in liver microsomes from both sexes of rainbow trout and channel catfish (Hansson and Gustafsson, 1981; Schlenk et al., 1993).

For estradiol metabolism, conversion has been shown to be dependent upon oxygen, which again strongly implicates the involvement of *P*450 enzymes (Hansson and Rafter, 1983). Characterization of estradiol metabolism by scup (*Stenotomus chrysops*) liver microsomes has revealed that major metabolites included the anticipated estriols and estrone. However, the principle catabolite was 2-hydroxy-estradiol (Snowberger and Stegeman, 1987), demonstrating that, as is the case for mammals, the enzyme estradiol 2-hydroxylase is involved. Liver 6β-hydoxylase activity (Klotz et al., 1986) and estradiol 2-hydroxylase activity are CYP1A1 (*P*450) enzymes distinct from the form (*P*450E) that

is inducible with hydrophobic organic xenobiotics or model compounds such as β-napthaflavone (Snowberger and Stegeman, 1987).

Distinct differences between the sexes can be observed for several *P*450 enzyme activities involved in steroid metabolism (Andersson and Foerlin, 1992; Stegeman, 1993). For example, in scup (*S. chrysops*) and winter flounder (*Pseudopleuronectes americanus*), estradiol 2-hydroxylase is present at higher levels in male than female liver microsome extracts (Snowberger and Stegeman, 1987), and activities of 6β-hydroxylase are lower in females than males of winter flounder, but not scup (Gray et al., 1991). In winter flounder, both of these activities can be suppressed by estradiol treatment, but these differences are reduced or not apparent when activity is normalized to total *P*450 protein levels, suggesting that factors other than differential production of CYP proteins may be responsible for this difference (e.g. competition for ribosomes by the stimulation of vitellogenin synthesis) (Snowberger and Stegeman, 1987; Gray et al., 1991). Similarly, the xenobiotic metabolizing *P*450 enzyme CYP1A1 is also found at lower levels in maturing female than male flounder (*P. americanus*), and measurements of mRNA levels indicate that this effect is mediated by a pretranslational regulation, again suggesting that access to protein synthetic machinery may play a role (Elskus et al., 1992). In kidney, male trout have dramatically higher kidney *P*450 6β- and 16β-hydroxylase activities than do females during the late reproductive stages, whereas females have a higher level of estradiol 2-hydroxylase (Andersson, 1990). Differences in kidney steroid enzyme levels between sexes may be caused in part by the presence of a sex-specific *P*450 enzyme (KM2) that is inducible by androgens, including 11-ketotestosterone (Andersson, 1992). Clearly, complex differences in enzyme type, level, and tissue distribution exist between the sexes at different reproductive stages, and thus, the exact role that these sexually dimorphic activities play in sex-steroid metabolism and sex differentiation remains unclear at present.

5. Sex-determination systems

The gonads of fish generally are very labile with respect to sex determination, but once a particular developmental profile has been selected by intrinsic controls, or directed by exogenous factors such as hormones, the state of gonadal differentiation may then be stably perpetuated throughout subsequent development. As discussed previously, exceptions to this stability exist in fish, exemplified by the ability of some hermaphroditic species to alter the course of sex differentiation (see Sections 3.2 and 5.7). However, these are specialized (albeit not rare) cases where sex determination is altered to allow flexible production of gamete types to maximize fitness. The stability of sex differentiation in gonochoristic species implies that sex-determination events primarily function during early development to set the course of gonadal development, and subsequent maintenance of the differentiated state is accomplished through the stability of gene expression patterns and feedback mechanisms to ensure a consistent profile of cellular and hormonal signals.

Several levels of control for gonadal determination seem feasible for fish, including intrinsic cell-autonomous genetic mechanisms, or endocrine, paracrine, behavioural, or environmental signals. For example, it is possible that PGCs interpret internal genetic or external environmental cues and directly transform into spermatogonia or oogonia; in turn,

the surrounding somatic cells would be induced by the germ cells to differentiate accordingly to provide an appropriate hormonal environment for further gonadal differentiation. Alternatively, somatic cells may initially interpret genetic or other sex-determination signals, and subsequent differentiation of PGCs occurs in response to signals derived from surrounding cells. In model genetic organisms, cell transplantation experiments and mosaic analysis have allowed researchers to determine whether sex determination occurs on a cell-by-cell basis or is based on interactions between adjacent or distant cell types. In *Drosophila* for example, chromosome loss or mitotic exchange can create animals with mixtures of genetically male and female cells, revealing that sex is determined mosaically on a single-cell basis (Cline and Meyers, 1996). Evidence from mammalian systems has strongly implicated that somatic cells rather than germ cells are the site of initial sex determination (Capel, 1996, 1998).

Fish utilize a wide array of mechanisms to control sex differentiation (Fig. 1). Many species use genetic systems that determine sex at fertilization, some use intrinsic clues provided by behavioural interactions among conspecifics, whereas others utilize changes in environmental factors such as temperature, season, or year class (Section 6). In theory, organisms may move between these different systems of sex determination without difficulty (Bull, 1985), depending on the degree of selection acting on the process, and in many cases, an equal ratio of males and females will be selected for in populations (Fisher, 1930). Indeed, for fish, experimental data has been provided supporting the concept that genetic selection will tend to balance sex ratio in *Menidia menidia* (Conover and Van Voorhees, 1990; Conover et al., 1992) and in *Xiphophorus maculatus* (Basolo, 1994, 2001).

The previous sections outline complex developmental, biochemical, and endocrine systems that have been found to influence sex differentiation in fish. Yet, these pathways currently do not provide complete insight into the initial events that ultimately destine an embryonic cell or gonad to proceed down male, female, or intersex pathways of development. Similarly, while the following sections outline described systems of sex determination in fish, as yet, we do not know at what biochemical, cellular or physiological level the developmental fate of the gonad is determined, nor do we know for fish whether sex determination is initiated first in germ cells or somatic cells, or both simultaneously.

5.1. Genetic determination of sex

The sex determination pathway can be viewed as a complex series of interacting biochemical processes that ultimately lead to sex-cell determination and differentiation. The activity or potency of each step in this pathway for determining sex will vary among individuals within a population, arising both from allelic variation at genetic loci encoding components of the pathway and from environmental influences (see Section 6). In genetic systems, certain components, or combinations of components, within the pathway may become dominant in influencing the direction of sex determination such that environmental factors have little influence. Some genes may direct ovarian and others testicular development, and the sex of a particular individual will be determined by the strength of the genetic factors it receives from its parents. Initially in the evolution of a sex-determining system, it is suspected that genetic effects would be polygenic, arising from the sum total influence of all genetic factors involved in sex determination in the genome.

However, over time, one component may gain such influence over the direction of the pathway that other genetic loci do not override its effects. In this case, sex will be determined by a simple, single-locus genetic system, and sex chromosomes may develop (see Section 5.2 and 5.3). In genetic systems, mechanisms range from purely polygenic controls, to those with dominant sex-determining factors mixed with autosomal controls, to highly evolved sex chromosomes with heterogametic (XY) males or heterogametic (ZW) females (Bull, 1983). Among fish species, all of these different approaches to controlling sex determination are utilized (Fig. 1).

The nature of genetic variation that leads to variable effects on sex determination in fish is currently not known. Different alleles will exist in populations that alter abilities of biochemical components to accomplish their respective tasks, and small effects are expected to act at a variety of levels, including biochemical conversions, signal reception and transduction, and activation or repression of gene complexes involved in initiating or maintaining the sex-determination cascade. As a hypothetical example, control of estradiol synthesis could play a key role. During the course of normal development, steroid-producing cells in the indifferent gonad may be directed to develop pathways for conversion of pregnenolone to testosterone even in the absence of any influence by sex-determination factors. In cells that are genetically female, the enzyme aromatase may be activated along with the other steroidogenic enzymes, whereas in genetically male cells, the aromatase gene remains inactive (either by repression or failure to activate). In mammalian systems, the product of the male-determining *Sry* locus has been shown to directly interact with regulatory regions of the aromatase gene promoter (Haqq et al., 1993). Although *Sry* is thought to be primarily expressed in Sertoli cells, this may not be exclusively so, and sequence variation in the aromatase gene promoter, or in SRY-like proteins capable of disrupting such interaction, could render the aromatase gene to be constitutively expressed or permanently repressed, resulting in altered estradiol production and gonadal development. Other genetic variation might influence aromatase catalytic enzyme activity to modify the ratio of estradiol to testosterone, or changes in the estrogen receptor could affect hormonal signaling. Because sex determination in gonochorists acts as a switch to initiate a developmental cascade that is stably maintained, even small genetic differences that influence this decision early in the process may ultimately have striking effects on sex determination (i.e. resulting in biased sex ratios, hermaphroditism, or sex inversion). Whatever the exact genetic influences utilized within a species, they must initiate or direct a cascade of gene regulatory controls that provide cells with specific differentiated sexual phenotypes. It is anticipated that genes upstream in developmental pathways may be more evolutionary diverse or variable than those in downstream regulatory positions (Marin and Baker, 1998) since gene mutations that gain control of the entire sex-determination process by interceding at the beginning of the pathway will cause fewer disruptions than would novel genetic activities which control only a downstream portion of sex differentiation.

The preceding illustrative examples are only hypothetical mechanisms that could be used to determine sex in fish, and in fact, no genes are currently known with certainty that are involved in the initial sex-determination process in fish. In *Drosophila* and nematodes, complicated pathways have been elucidated that regulate sex determination (Cline and Meyers, 1996), but these do not share homologies with vertebrate systems except for the DM domain proteins (described below). In vertebrate systems, the H–Y antigen (the

product of the *Smcy* locus) was originally believed to be a good candidate for a sex-determining factor since it was associated with tissues of the heterogametic sex in mammals and some other vertebrates (Simpson et al., 1997; Wolf, 1998), but further studies have shown that, in fish, this antigen is not differentially present between the sexes or clearly associated with heterogamety in all species (Muller and Wolf, 1979; Nakamura et al., 1984; Koehler et al., 1995), and is more associated with the gonadal phenotype (examined in sex-reversed fish arising naturally in hermaphrodites or by experimental hormone treatments) than genetic factors (Pechan et al., 1979, 1986; Duchac and Buehler, 1983; Reinboth et al., 1987). These observations suggest that H–Y antigen probably plays a secondary role in primary sex determination, but may well be very important for subsequent differentiation or redifferentiation. Indeed, many genes that are expressed in a sex-specific fashion (i.e. aromatase, *DMRT1*; see below) may be a consequence of sex differentiation rather than a causal (i.e. sex determining).

For mammals, the Y-linked gene *Sry* has been characterized and definitively shown to be the sex-determination locus (see above). An *Sry* antagonist (*Dax1*), and several other autosomal genes (e.g. *Sox9*, *SF1*, *Wt1*, *Lim1)* involved in steroid-cell differentiation and gonadal differentiation in mammals have also been characterized (Swain and Lovell-Badge, 1997; Capel, 1998; Koopman, 1999). SRY is a phosphorylated nuclear protein that appears to interact with LEF1-like transcription factor binding sites to alter the bending of DNA, which may lead to changes in chromatin domain architecture and transcription. *Sry* is expressed in Sertoli cells, and may act in a dosage-sensitive fashion by repressing other negative regulators (i.e. *Dax1*) of testis differentiation (McElreavey et al., 1993; Koopman, 1999). Overexpression of *Dax1* gene product (of the nuclear hormone receptor family), either in natural chromosomal duplications in humans or by transgenesis in mice, counters the testis-determining actions of SRY, whereas absence of DAX1 in mice has no effect on ovarian development (Goodfellow and Camerino, 2001). This strongly suggests that DAX1 is an antitestis determining factor. In mammals, the absence of SOX9 function can result XY sex reversal, and *Sox9* gene expression is upregulated in male and down-regulated in female genital ridges just prior to sex differentiation, suggesting an important role for this protein in testicular development (Koopman, 2001). Recently, homologues to *SF1* and *Sox9* have recently been characterized from fish (Fukada et al., 1995; Ito et al., 1995, 1998; Vriz and Lovell-Badge, 1995; Santacruz and Vriz, 1996; Koyano et al., 1997; Liu et al., 1997; Takamatsu et al., 1997; Kanda et al., 1998; Yamashita et al., 1998; Zhou et al., 2001) and have been found to be members of the same high-mobility group (HMG) family of DNA-binding proteins. One member of this group (*Sox-9*) does show differential expression between male and female gonads in tilapia (Nagahama, 1999) as is found in mammals and birds (Kent et al., 1996; Morais da Silva et al., 1996), consistent with an important role for testis development in all vertebrate groups.

Remarkably, another gene family involved in sex-determination in invertebrates also appears to be involved in vertebrate sex determination. Proteins from this gene family contain DM-domains [a motif identified by homology between genes involved in sex determination in insects (*Dsx*) and nematodes (*mab3*)] with DNA-binding characteristics. One member of this group, *DMRT1*, has been found to contain an additional male-specific motif homologous to the male splicing variant of *Drosophila Dsx* and is expressed primarily in a testis-specific fashion in several vertebrate groups (Raymond et al., 1998).

In tilapia, *DMRT1* homologues (*tDMRT1*) possess the male-specific motif found in DSX, and expression appears is testis (Sertoli cells) specific (see Fig. 6), whereas another DM homologue (*tDMO*) lacked this motif and was found expressed only in ovary (Guan et al., 2000). Interestingly, examination of upstream sequences reveal SRY binding sites within *tDMRT1* but not *tDMO*, suggesting a close linkage between *Sox* and *DMRT1* gene products in sex determination pathways. In trout, *rtDMRT1* is also expressed predom-

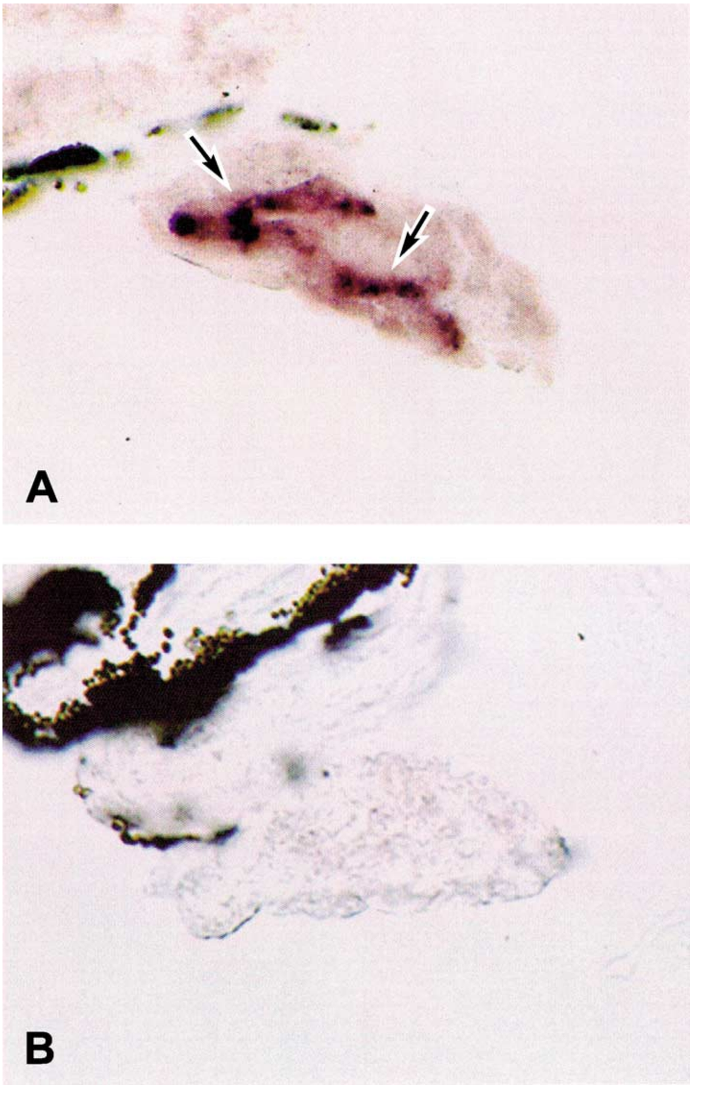


Fig. 6. Male-specific expression of *DMRT1* in tilapia gonad during sex differentiation (15 days posthatching). (A) Male (XY), (B) Female (XX). *DMRT1* is found to be expressed (arrows) only in somatic (Sertoli) cells of XY gonads. In situ hybridisation. (Unpublished, provided by T. Kobayashi).

inantly in the testis compared to ovary (> 10 fold) (Marchand et al., 2000). Masculinization of tilapia genetically female (XX) gonads with androgen induces *DMRT1* expression, whereas treatment of genetically male (XY) trout with estradiol and genetically female (XX) fish with the androgen 11β-hydroxyandrostenedione both resulted in reduced expression (Guan et al., 2000; Marchand et al., 2000). These data suggest that *DMRT1* genes are expressed in response to testis differentiation induced by other factors (i.e. steroids), and, in these species, are either located somewhat downstream in the sex determination pathway as is the case for *Dsx* and *mab3* in invertebrates (Marin and Baker, 1998), or they are sensitive to feedback regulation. Accordingly, *DMRT1* expression in XX testis is consistent with this gene not being sex linked in three fish species (tilapia, rainbow trout, and medaka) (Guan et al., 2000; Marchand et al., 2000; Brunner et al., 2001); however, other as yet unidentified members of the DM gene family may play important primary roles in sex determination in fish.

Based on their expression patterns and association with sex determination in other species, the gene families described above are good candidates for genes involved in this process in fish. Indeed, a strong candidate for the sex-determining gene has been recently identified on the Y chromosome of medaka by positional cloning and detailed sequence analysis of BAC clones (Matsuda et al., in press). This gene (*DMY*) also is a member of the DM-domain gene family, and genetic evidence indicates it is located only on the *Y* chromosome in a region that is essential for male germline development. *DMY* is expressed in male somatic gonadal cells at the time of initial sex determination (analogous to *Sry* described above), and provides the first example of a sex-determination gene in lower vertebrates. In the future, characterization of similar genes from other vertebrates, as well as direct analysis of fish genomes, will undoubtedly provide a rich source of material for investigation of sex determination in fish.

5.2. Cytogenetic evidence for sex chromosomes in fish

More than 1700 species of fish have now been cytogenetically characterized (Arkhipchuk, 1995), of which approximately 176 species or 10.4% have been found to have cytogenetically distinct sex chromosomes (Appendix B). Heteromorphic chromosomes have been observed between the sexes in at least 72 families within Chondrichthyes and Osteichthyes, but none have yet been identified in Aganthans where evidence exists for nongenetic sex determination (see Section 6.2). These figure represent a minimum estimate for sex chromosome frequencies in fish since not in all cytogenetic studies have sex chromosomes been specifically looked for (due to absence of both sexes for analysis, or the phenotypic sex of samples is unknown). Perhaps more importantly, cytogenetic differences between heteromorphic pairs may in many cases be too small to be observed by current cytogenetic techniques [fish chromosomes are in general small (Gold et al., 1980)]. The examination of chromosome pairing in synaptonemal complexes can reveal the presence of sex-chromosome regions that are otherwise not visible in somatic chromosomes (Carrasco et al., 1999), but such techniques are only rarely applied in fish karyotype analysis (Oliveira et al., 1995; Van Eenennaam et al., 1998a; Campos-Ramos et al., 2001). An alternative estimate of the frequency of sex chromosomes in fish can also be provided by examining the number of species (170) where sex chromosomes were not found in studies where they were

specifically searched for (see Appendix B, ND = not detected). Using this approach, the fraction (176/176 + 170) of species found with sex chromosomes is much greater, 50.1%. Although this undoubtedly represents too high an estimate (since once sex chromosomes have been found researchers will tend to examine related species), it nevertheless suggests that the occurrence of sex chromosomes among fish species may be somewhat higher than indicated by the simple frequency observed among all known fish karyotypes. Currently, more than twice as many species display male heterogamety compared to female heterogamety (see Appendix B). It has been hypothesized that XY systems of sex determination are favoured to evolve in species where fitness positively correlates with male size, whereas ZW systems occur with female size advantage (Kraak and de Looze, 1993).

As in other organisms, sex chromosomes in fish take many karyotypic forms, consisting of subtle changes or major chromosomal rearrangements. Cytogenetic differences detected between sex chromosomes include: additions or deletions of heterochromatic blocks (e.g. Phillips and Ihssen, 1985; Andreata et al., 1992; Cano et al., 1996; Stein et al., 2001), reductions in chromosome size (e.g. Park and Kang, 1979), increases in chromosome size (e.g. Galetti et al., 1981), and chromosome rearrangements (e.g. Uyeno and Miller, 1971; Thorgaard, 1978; Bertollo et al., 1983; de Almeida Toledo et al., 1984; Pezold, 1984; Vitturi et al., 1991b). Similar differences can be found with other chromosome pairs between individuals within a population, but when these differences in chromosome structure are restricted to one sex, the presence of sex chromosomes is strongly indicated. Karyotypic analysis reveals which sex is heterogametic and which is homogametic, thus providing information about the sex-determination mechanism employed by the species. The average sex-chromosome length in fish has been estimated to be approximately 5% of the total karyotype (Arkhipchuk, 1995), which is somewhat larger than the average chromosome size. This suggests that, on average, degeneration of sex chromosomes in fish has not usually proceeded to such a degree that large portions of the chromosome have been lost or severely genetically damaged (see below).

Among species with male heterogamety, several karyotype systems have been discovered (Appendix B). XY systems are those where the X and Y chromosomes are cytogenetically distinguishable in some way, but the diploid chromosome number ($2n$) between the sexes is the same (e.g. Thorgaard, 1977; Phillips and Ihssen, 1985). XO systems are those where the Y chromosome has been lost (e.g. Chen, 1969), and males possess one less chromosome than females. In multiple chromosome systems of the type $X_1X_2X_1X_2/X_1X_2Y$, the Y chromosome has become attached to an autosome by translocation or Robertsonian fusion, and consequently males and females have different diploid chromosome numbers and often different numbers of metacentric and acrocentric chromosomes as well (e.g. Uyeno and Miller, 1971; Thorgaard, 1978). In XX/XY_1Y_2 systems, an acrocentric X chromosome has become fused with an autosome (Frolov, 1990). Analogous chromosomal systems have been observed (Appendix B) for species with female heterogamety (Moreira et al., 1985; Sharma and Tripathi, 1988; Koehler et al., 1995; Ueno et al., 2001).

5.3. Evolution and plasticity of fish sex chromosomes

Genes involved in sex determination (see Section 5.1) can be distributed throughout the genome, located on single chromosomes, or restricted to a single genetic locus. In

polygenic systems, sex-determining loci on different chromosomes can segregate randomly to offspring and provide a range of influences on the sex-determination process, and, in this case, sex is determined by the cumulative genetic actions of all sex factors received by an individual rather than by the allelic state at any one locus (Bull, 1983). The condition of polygenic sex determination may persist until a strong sex-determining factor evolves in the population, at which time a single genetic locus may, temporarily or permanently, come to dominate the process. Thus, single locus control restricts sex to be determined by a single chromosome (the Y or W chromosome; see below) where the genetic state of any other chromosome in the genome has little effect on the outcome of sex determination. In populations, this is undoubtedly a dynamic process whereby sex chromosomes and other polygenic factors increase and decrease in their influence over the sex-determination process, and control moves back and forth between monogenic and polygenic control. Indeed, polygenic systems have been hypothesized to evolve from systems with clear evidence of sex chromosomes (apparent in related species) (Ota et al., 2000), suggesting that the number and type of loci involved in sex determination in fish can be very fluid.

Depending on the nature of a genetic factor that has gained dominance in the sex-determination process, either male-specific Y chromosomes or female-specific W chromosomes can result (Bull, 1983). Sex-limited chromosomes (both Y or W) can arise by two major forms of gene mutation. In the first, gain-of-function mutations may arise on a chromosome to direct the course of sex differentiation. Such mutations may be hypermorphic (elevated activity) or neomorphic (new function) in nature, and are usually dominant or co-dominant in their genetic action. Hence, in these cases, it is the presence of a Y or W chromosome that determines sex. An alternative mode of sex chromosome formation can occur when mutations in sex-determination genes result in reduced gene function (hypomorphs). In this case, dosage-dependent amounts of a gene products from sex determining loci on sex chromosomes (either X or Z, respectively) in the homogametic sex influence the course of sex differentiation. In both cases, the genetic changes lead to the presence of a single chromosome that is found only in that sex in populations. It is the isolation of such chromosomes in one sex that leads to the remarkable cytogenetic transformations that sex chromosomes can undergo (Lucchesi, 1978; Charlesworth, 1991).

All chromosomes within a genome are subjected to mutational damage from exogenous chemicals or radiation, and from intrinsic sources (e.g. free radical damage, errors in DNA repair, transposition or exchange events, etc.), and, in many cases, such mutations will disrupt the function of adjacent genes. In polygenic sex-determination systems, deleterious recessive mutations arising on chromosomes possessing sex-determining loci can be eliminated from populations by selection against homozygotes as occurs for other chromosomes. In this case, the sex-determination processes would not be seriously hampered since undamaged homologous chromosomes could still participate in sex determination among individuals. However, in cases where the number of chromosomes (or loci) involved in sex determination is few, the population genetic effects of mutation on each individual chromosome becomes more pronounced. In the extreme, in systems involving single chromosomes, the sex-determining chromosome (W or Y) exist only in heterozygous condition (XY or ZW) and cannot, under normal circumstances, become homozygous in populations. Thus, unable to experience the same negative selection in homozygous form, sex-specific Y or W chromosomes can accumulate recessive genetic

modifications, whereas similar mutations occurring on homologous chromosomes can become homozygous (in XX females or ZZ males) where selection is able to act and eliminate chromosomes with reduced fitness.

The types of mutational damage acquired by sex chromosomes can be in the form of single point mutations, deletions or duplications of small or large DNA regions, rearrangements with other chromosomes, amplifications of repetitive sequences, orinsertions of autosomal DNA or mobile transposable elements (see Section 5.4). If this process proceeds for many generations, sufficient genetic alterations may occur that allows detection of morphological differences at the cytogenetic level in the form of a heteromorphic pair of sex chromosomes. Indeed, the presence of a cytogenetically distinct sex chromosome in a species implies that the sex-determination system has been stable for some time.

Mutation of loci on sex chromosomes will most often result in a reduction in the amount of gene product synthesized per cell, but this may have only a small effect on the total amount of substrate converted (Kacser and Burns, 1981) due to the nonlinear relationship between gene product level and total flux through biochemical pathways (hence the recessive nature of most mutations). While the physiological effects of each such reduction is small, the normal diploid level of gene product probably provides optimum fitness in nature, and small impairments of gene action occurring at many loci on sex-limited chromosomes would eventually accumulate such that fitness is impaired. In some vertebrate and invertebrate species, dosage compensation mechanisms have evolved to compensate for the reduction of gene function occurring on sex-limited chromosomes (Lucchesi, 1978; Charlesworth, 1996; Pannuti and Lucchesi, 2000). Thus, these mechanisms can promote the development and maintenance of sex chromosomes in populations by reducing the negative effects on fitness arising from mutation accumulation. To date, no evidence exists that dosage compensation mechanisms have evolved in fish, which may explain in part why sex-chromosome evolution in fish has remained very plastic with no single mechanism predominating.

Genetic damage can accumulate on chromosomes that do not actually encode sex-determining genes, but are nevertheless restricted to one sex by virtue of homology to, and meiotic pairing with, chromosomes that determine sex based on dosage or gene balance relative to the autosomes (i.e. *Drosophila* and nematodes). In such cases, since no gene function is actually required from the sex-limited chromosome (particularly if dosage compensation is operating—see below), degeneration can proceed in some cases until the chromosome is actually eliminated from the population, resulting in an XX/XO (or ZZ/ZO) system (see Section 5.2).

In populations, individual Y or W chromosomes will accumulate different profiles of genetic damage, and the frequencies of an individual sex chromosome will fluctuate due to population genetic processes (genetic drift, bottlenecks, selection, etc.). Over time, particular Y or W chromosomes may become fixed, and be associated in the population with all individuals of the sex determined by that chromosome. Subsequently, such sex chromosomes will acquire new genetic damage and again be subjected to new fixation forces. This repeated process of mutation and fixation is termed Muller's ratchet (Lucchesi, 1978; Charlesworth, 1991), and may occur within a population such that significant mutational damage accumulates on the sex chromosome. This damage will be

most pronounced in the vicinity of the sex-determination locus, where mutations have reduced chance of recombining onto the homologous sex chromosome where selection and elimination may occur. However, accumulation of genetic damage may also involve entire chromosomes where genetic exchange has been restricted by disruptions in recombination or by the presence of inversions or other chromosome rearrangements that interfere with chromosome pairing (Lucchesi, 1978). In nature, several variants of a sex chromosome probably exist in fish populations, each in various stages of fixation or elimination. It is likely that competition among sex chromosomes, based on both negative and positive fitness effects they carry, is commonplace.

Many fish species do not possess cytogenetically visible sex chromosomes, yet some of these same species are known to utilize genetic determination systems that are associated primarily with single chromosomes (see Appendix B and Sections 5.4–5.7). It is possible that in fish, the strength of sex-determining factors on the Y or W chromosome are not particularly strong relative to the effects of sex factors on the autosomes. If so, during the evolution of fish, it is very likely that new mutations (at loci on autosomes) might regularly have arisen that were capable of overriding the effect of the existing sex chromosomes (Komen et al., 1992b,c) (see Section 5.7.1). In such cases, XY females or ZW males would appear in populations, and in matings with regular individuals, could produce YY or WW individuals. If these sex chromosomes carry even single lethal mutations (acquired as described above), the chromosomes would be quickly removed from the population by selection. The former autosome that harbours a new mutation controlling sex determination will hence become the new sex chromosome, and the process of chromosomal degeneration will begin anew. It is interesting to note that species which possess autosomal influences on sex determination (e.g. tilapia species; Majumdar and McAndrew, 1986), or which have sex determined by environmental or other cues (see Section 6), do not usually possess cytologically distinct sex chromosomes, possibly because any given chromosome does not retain a dominant influence over sex determination for a sufficiently long period to undergo significant molecular degeneration. Differences between the sex chromosomes may be quite subtle and limited to differential pairing in meiosis, and this approach has been used to identify putative sex chromosomes in tilapia species with XY (*O. niloticus*) and ZW (*O. aureus*) systems (Carrasco et al., 1999; Campos-Ramos et al., 2001). Indeed, analysis of *O. aureus* has indicated that two separate pairs of sex chromosomes may be present, consistent with the complex pattern of genetic sex determination observed among intra- and interspecific crosses (see Section 5.5). This plasticity of sex chromosomes also implies that, among the many fish species, sex-determining genes on Y or W chromosomes may well be different, although each is probably operating within the same sex-differentiation control pathway.

Sex chromosome systems have been found to be polymorphic within a species, indicating the dynamic evolutionary nature of these systems. Thus, in rainbow trout, heteromorphic chromosomes differ among populations and are identifiable in males of only some populations (Thorgaard, 1977, 1983b; Frolov, 1989). Similarly, a Y-autosome translocation is found only in some males of *Blennius tentacularis* (Carbone et al., 1987). Interestingly, in *C. julis*, a protogynous species showing behaviourally influenced sex determination (see Section 6.3), a polymorphic Y chromosome has been identified in individuals developing as primary males, but this sex chromosome is absent in males arising from natural sex

inversion of females (Duchac et al., 1982). In this species, females and secondary males are homogametic and have sex determined by behavioural mechanisms, whereas in primary males, a genetic system appears capable of overriding social control systems. This situation, where both genetic and labile (hermaphroditic) sex-determination systems are indicated in the same species, appears to be quite rare among fishes (compare Appendices A and B). The separation of genetic and other sex-determination mechanisms (see above) extends to higher relationships among fish species: Only approximately 20% of families with sex chromosomes also have genera with sex-changing species. Sex chromosomes are not anticipated to become karyotypically differentiated to any great extent in species that do not have a firm system of genetic sex determination. If other mechanisms are able to override this process, such that matings can occur between two heterogametic individuals, this will allow sex chromosomes to become homozygous in populations and become eliminated if substantial chromosomal alteration has occurred. In these situations, one anticipates selection to strengthen one type of sex-determination mechanism at the expense of the other, or, in species like *C. julis* (Duchac et al., 1982), a balance may be established where two different reproductive approaches are maintained within breeding groups as an evolutionary strategy. For one order of fish, the Aulopiformes, a combination of karyotypic and molecular phylogenetic analysis has revealed that sex chromosomes can indeed be lost in the course of evolution, since a hermaphroditic species was found to be derived from gonochoristic species with well-differentiated sex chromosomes (Ota et al., 2000).

5.4. Genetic and molecular evidence for sex chromosomes

Sex chromosomes were first inferred from classical genetic analysis through observation of phenotypic marker segregation to the sexes in *Drosophila melanogaster*. For example, in XY systems, genetic markers that segregate from fathers to daughters are located on the X chromosome, whereas markers segregating to sons are Y linked. Markers in female parents segregate to both sexes, but recessive markers may be uncovered in the case of hemizygosity of the X chromosome in XY males. The complementary case where markers segregate from mothers to only daughters implies a ZW system of sex determination. Such inheritance patterns reveal the mode of genetic sex determination in a species (XY or ZW), and, depending on frequencies of genetic recombination between the sex-determination locus and the marker, can also indicate whether genetic linkage extends to an entire chromosome or just to the region surrounding the sex-determination locus. In fish species, genetic markers used in such segregation analyses have been both phenotypic and molecular, the latter including both protein and DNA variant loci.

5.4.1. Phenotypic markers

Classical studies examining sex linkage of phenotypic markers (Yamamoto, 1969) in fish revealed that sex was determined by an XY system in *Oryzias latipes*, *Poecilia reticulata*, *Poecilia nigrofasciata*, *B. splendens*, and five species of *Xiphophorus* (*X. variatus*, *X. xiphidium*, *X. couchianus*, *X. milleri*, *X. montezumae cortezi*) including some strains of *X. maculatus* (Aida, 1921; Wing, 1922; Yamamoto, 1969). Detailed investigations with *Xiphophorus* species were particularly revealing, since it was found that both male (XY) and a female (ZW) heterogametic systems of sex-determination existed among species in

the genus, as well as in different strains within the single species *X. maculatus*. By performing hybridization experiments between male and female heterogametic *Xiphophorus* species, and among *X. maculatus* strains (Bellamy, 1936; Gordon, 1946, 1947; Kallman, 1965), it was concluded that the Z and Y chromosomes are equivalent, and contain sex-determining factors that are epistatic to those found on the X chromosome. The W chromosome was found to be distinct, and female factors located thereon are epistatic to male determining factors on the Y or Z chromosome (thus WX, WY, and XX individuals are female, but only XY and YY individuals are male). To maintain this system within the species, modeling has indicated that the fitness of WY and WX females (e.g. fecundity) must be greater than XX females, and the fitness of XY males must be better than YY males for characters such as mating success (Orzack et al., 1980). Most importantly, these early studies on sex-linked marker segregation in model species were the first to reveal the complex and plastic nature of sex-determination systems that exist in fish.

Specific phenotypic markers affecting skin pigmentation have been identified in several fish species where male phenotype is important for mate selection by females. In the guppy *P. reticulata*, both the black caudal peduncle (*Bcp*) and red tail (*Rdt*) markers have been found to be dominant Y-linked traits (Fernando and Phang, 1989, 1990; Khoo et al., 1999), as have orange pigment spotting loci (Houde, 1992) and the *Nigrocaudatus II* gene affecting body pattern (Nayudu, 1979). In medaka, which possesses an XY sex-determination system, a marker affecting leucophores is tightly sex linked (Wada et al., 1998), and sex-linked pigment pattern genes have been mapped on the *Xiphophorus* genome map (Morizot et al., 1991). A Y-linked spotting pattern gene has also been characterized in *Gambusia affinis* mosquitofish (Angus, 1989).

Traits affecting morphological characters and fitness traits have also been found to be sex linked in fish. For example, a sex-linked low-temperature resistance gene has been identified in *P. reticulata* (Fujio et al., 1990), and in *X. maculatus*, a sex-linked gene controls the age of onset of sexual maturity (Kallman and Borkoski, 1978; Schreibman and Kallman, 1978). In *X. nigrensis* males, size and age at maturity are influenced by a Y-linked locus that can alter mating success in field and laboratory populations (Morris et al., 1992), but interestingly, overall fitness does not differ between the size morphs (Ryan et al., 1992). However, this strategy seems to be somewhat plastic, as other poeciliids such as *Phallichthys quadripunctatus* do not possess this sex-linked genetic trait (Kolluru and Reznick, 1996). Tumor-inducing loci have also been extensively studied in *X. maculatus* (see below), for which some are sex linked (e.g. Ahuja et al., 1979).

In studies examining segregation of phenotypic markers, it is critical to distinguish between traits that are encoded by sex-linked genetic loci and those that arise as secondary-sex characteristics that appear only in one sex because of hormonal or other influences.

5.4.2. Protein markers

Isozyme loci have also proven very useful for the identification of sex-linked modes of inheritance in fish. In channel catfish, the *GPI-B* locus is found on the sex chromosomes, approximately 16 map units away from the sex-determination locus and on the other side

of the centromere (Liu et al., 1996a). In rainbow trout, isozymes have been extensively studied, but only the *HEX-2* and *sSOD-1* loci have been found to be sex linked (Allendorf et al., 1994). In charr, the *EST-2* locus in *S. alpinus* appears to be linked to the sex-determination locus in some populations (all males are heterozygotes) (Nyman et al., 1984), and a male-specific IDH isozyme variant has been described for *G. aculeatus* (Withler et al., 1986). Using hybrids between *S. alpinus* and *S. fontinalis*, studies have revealed that the *LDH-1*, *AAT-5*, and *GPI-3* loci are genetically linked to the sex-determination locus (May et al., 1989). As is the case in channel catfish, the markers map to the other side of the centromere from the sex-determination locus, and because they do not segregate with sex in other char species, sex linkage in *S. alpinus* may have been acquired by a species-specific translocation event that fused an autosome and a sex chromosome. Analysis of sex linkage is not restricted to isozyme mobility variants: null alleles at the MDH-4 locus in *P. reticulata* show partial sex-linkage (Fujio and MacAranas, 1989).

As was mentioned for phenotypic markers, it is important to note that the presence of novel isozymes restricted to one sex does not necessarily indicate that the marker is sex linked, since sex-specific gene-expression patterns may be responsible in some cases (see Withler et al., 1986).

5.4.3. DNA markers

DNA markers provide useful tools for examining sex linkage in fish since DNA structure is not anticipated to change with altered physiology or environments. Further, examination of DNA sequence organization on sex chromosomes can provide important insights into the evolutionary processes that are operating to influence sex-chromosome structure, and ultimately, can yield information on the conservation (or lack thereof) of sex-determination processes among species.

Some genes that are associated with the Y chromosome in humans, including the mammalian sex-determining locus *Sry* and a closely linked gene *Zfy*, are present in fish genomes, but are not associated with the sex chromosomes in trout (Ferreiro et al., 1989), medaka (Fukada et al., 1995), channel catfish (Tiersch et al., 1992), *Anthias squamipinnis* (Wachtel et al., 1991), turbot (*Scophthalmus maximus*) (Husebye et al., 1994), and chinook salmon (Devlin, unpublished). Similarly, in *O. niloticus*, no sex-specific arrangement was observed for either *Zfy* or a sequence derived from the W chromosome of chickens (McConnell et al., 1996). These findings indicate that *Sry* and *Zfy* genes exist in piscine genomes and thus may play a role in sex-determination pathways, but probably they are not the primary signal used to control sex in genetically determined species.

In some cases, genes and/or tightly associated sequences have been cloned that provide DNA markers tightly linked to the sex-determination locus. In *X. maculatus*, sequences related to the *v-erbB* oncogene cosegregate with a sex-linked melanoma-forming locus (Zechel et al., 1988). Linked DNA markers near the sex-linked *Tu* locus are responsible for melanoma formation in *Xiphophorus* (Schartl, 1988), and further analysis revealed that the sex-linked *Xmrk* gene is duplicated in *Tu* mutant fish (Schartl, 1990; Schartl and Adam, 1992; Weis and Schartl, 1998). A rapid PCR-based sex test employing the *Xmrk* locus has been developed for *Xiphophorus* (Coughlan et al., 1999), and *Xmrk* locus has been mapped cytogenetically by in situ hybridization to the subtelomeric region of the long arm of the *X. maculatus* sex chromosomes (Nanda et al., 2000). In medaka, *O. latipes*, which possess an

XY system, a random DNA clone that is very tightly linked to the sex-determination locus has been isolated from an inbred strain. This marker is not sex-linked in all medaka strains, but the sequence appears to be conserved within the genus (Matsuda et al., 1997). Similarly, another sex-linked marker in medaka appears to be composed of repetitive DNA and is not found in all strains (Matsuda et al., 1998). These markers are genetically tightly linked to the sex-determination locus due to suppression of crossing over in males rather than due to tight physical linkage (Matsuda et al., 1999), a finding confirmed by detailed construction of a linkage map for this species (Naruse et al., 2000). In situ localization of markers linked to the sex-determination locus has allowed cytogenetic identification (Fig. 7A) of the Y chromosome in medaka (Matsuda et al., 1998), and, more recently, identification of the sex-determination locus itself (Fig. 7B).

In rainbow trout, two polymorphic DNA sequences located on the Y chromosome have been identified by RAPD analysis (Iturra et al., 1998). One sequence (P9) is present in all males and absent from all females in the Mount Lassen trout strain, but in another strain (Scottish), it can also be found in 62% of females. The other sex-linked sequence characterized was found in approximately 75% of males and no females in both strains examined. These findings clearly reveal the molecular diversity that exists on the Y chromosome of rainbow trout, findings which correlate with the observation that heteromorphic chromosomes can be identified in some but not all (Thorgaard, 1983b) rainbow trout strains (see Appendix B). The P9 probe has also been used in in situ hybridization studies with coho salmon (Iturra et al., 2001), where it was found to hybridize near a growth hormone sequence known to be sex linked in this species (see below).

Ribosomal gene sequences (NORs) have been identified cytogenetically on sex chromosomes in some fish (Li and Gold, 1991; Ren et al., 1993; Khuda Bukhsh and Datta, 1997). In rainbow trout, Atlantic salmon and arctic charr (Pendas et al., 1994; Reed and Phillips, 1997), in situ hybridization has not revealed a sex-specific organization of the NORs. However, a 5S rDNA gene cluster is linked to autosomal NORs in rainbow trout, but in addition, a 5S gene cluster has been detected in heterochromatic regions of the X chromosome (Moran et al., 1996). 5S sequences are also linked to the short arm of the Y chromosome in chinook salmon (Stein et al., 2001).

Molecular genetic genome mapping studies are beginning to provide a wealth of genetic markers for segregation studies. The sex chromosomes have been identified in a rainbow trout linkage map generated using a variety of microsatellite and AFLP DNA markers. This map, generated from doubled haploids, has located the sex-determining locus in a distal position on the sex chromosomes (Young et al., 1998). Similarly, for medaka, the sex chromosomes were identified in a detailed mapping studies and several DNA markers have been found to map closely with the sex-determination locus (Naruse et al., 2000; Sato et al., 2001). Two Y-chromosome linked AFLP markers have also been identified in the threespined stickleback (Griffiths et al., 2000), consistent with identification of sex-linked isozyme loci in this species (Withler et al., 1986).

As described above, degeneration of sex chromosomes can often be associated with the accumulation of repetitive DNA, pseudogenes, or transposable elements. A repeat sequence termed Bkm (isolated as a minor satellite DNA component from the banded krait snake genome) is comprised of simple tetranucleotide arrays (GATA and GACA). These sequences have been associated with the W and Y chromosomes of several nonpiscine

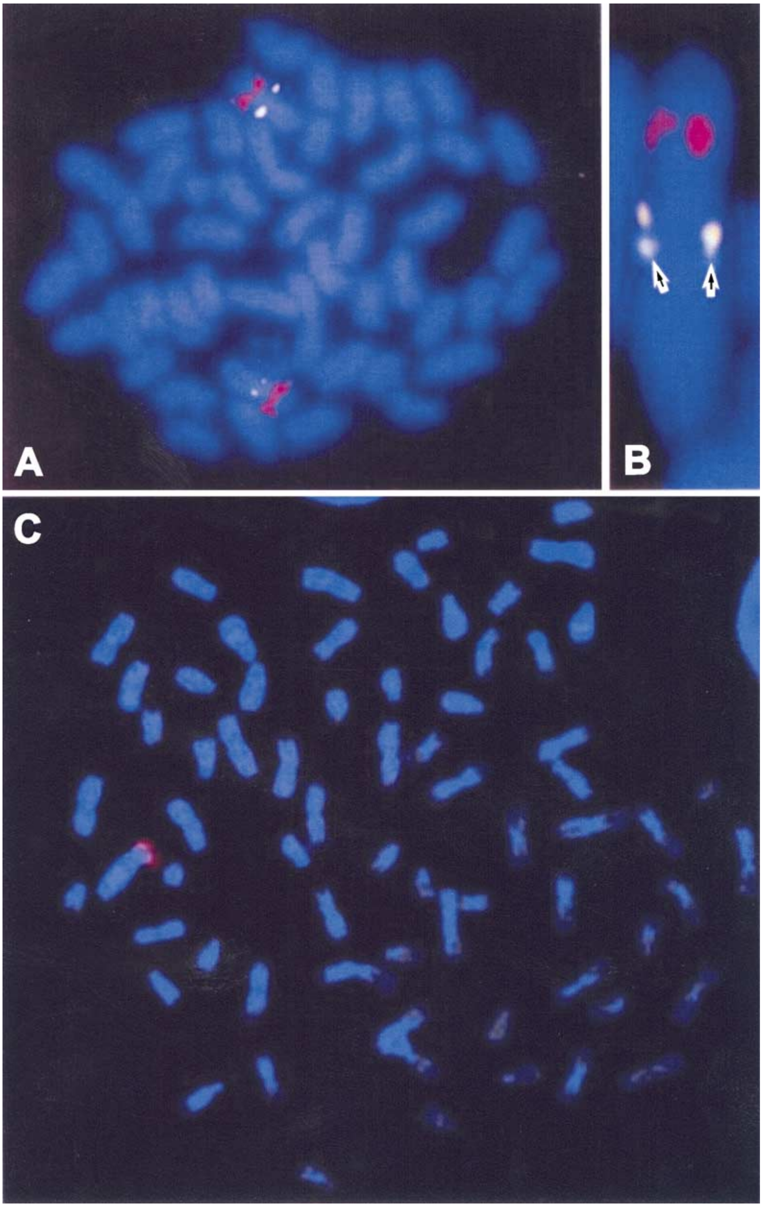


Fig. 7. Fluorescence in situ hybridization of fish metaphase chromosomes. (A) Sex-linked markers SL-1 (yellow) and Sl-2 (red) hybridize to both the X and Y chromosomes of medaka. (B) A medaka sex chromosome with three different probes (*SL2*, *SL1*, sex-determining region, *SD*). Signals of a BAC clone containing the sex-determining region are light blue. (Unpublished, provided by M. Matsuda et al.). (C) Identification of the Y chromosome in chinook salmon by in situ hybridization (red) of the Y-linked DNA marker OtY1. (From Stein et al., 2001, by permission of Karger Publishers, Bassel).

species, and have been used as probes to examine whether such repeats are also sex linked in fish. Although present in the genome, they do not appear to be organized in a sex-specific fashion in rainbow trout (Lloyd et al., 1989), channel catfish (Tiersch et al., 1992), mosquitofish *G. affinis* (Patel et al., 1993), turbot (Husebye et al., 1994), or the protogynous *A. squamipinnis* (Wachtel et al., 1991). However, indications that Bkm-like sequences can be associated with the heterogametic sex of several fish species, including *X. maculata* and rainbow trout, have also been reported (Ewulonu, 1987). GACA and GATA repeats have been found associated with the Y chromosome in *P. reticulata* strains (Nanda et al., 1990; Nanda et al., 1992), and other *Poeciliid* species were associated with other sex-specific repeat sequences (Nanda et al., 1992). Interestingly, *P. reticulata* obtained from the wild in Trinidad do not show any evidence of these repetitive DNA sequences on their sex chromosomes (Hornaday et al., 1994), suggesting that selective pressures influencing tolerance for these sequences in the genome may differ between wild and laboratory environments.

A microsatellite marker *Str-A9* has been found to be tightly associated with the sex-determination locus in brown trout *S. trutta* (Prodohl et al., 1994), and a satellite DNA sequence associated with telomeres and centromeres is located in an interstitial position on a neo-Y chromosome in the Antarctic icefish, *Chionodraco hamatus* (Capriglione et al., 1994). However, repetitive telomeric sequences (which are variable and located on the ends of all chromosomes) were not observed to be organized in a sex-specific fashion in channel catfish (Tiersch et al., 1992) or chinook salmon (Devlin, unpublished data).

Species in the genus *Leporinus* utilize a ZW system of sex determination (Galetti et al., 1995; Mestriner et al., 1995). *L. elongatus* has a W chromosome that is very large relative to the Z chromosome, suggesting that substantial accumulation of new sequences may have occurred. Subtractive hybridization has been used to isolate repetitive DNA sequences from the sex chromosomes of *L. elongatus* (Nakayama et al., 1994), and one DNA clone detects sequences found on both the Z and W chromosomes, whereas another is restricted to the W chromosome. By comparing gonadal phenotypes with cytogenetic and molecular results, these authors identified exceptional individuals where these three characteristics did not correlate. These exceptions all contained a W chromosome cytogenetically, but in one case, a phenotypic female lacked the W-specific DNA sequence (Nakayama et al., 1994). Such exceptional individuals may have arisen by recombination or deletion events on the sex chromosomes, and provide evidence of the fluidity of W sex-chromosome structure in fish at the molecular level.

Other repetitive DNA sequences have been isolated from piscine sex chromosomes. Lake trout *Salvelinus namaycush* contains a Y chromosome cytogenetically distinguishable from the X by the absence of a quinicrine bright heterochromatic band (Phillips and Ihssen, 1984, 1985), and microdissection of the Y chromosome yielded repetitive DNA sequences that were located on both X and Y sex chromosomes of lake trout (Reed et al., 1995). Interestingly, paint probes developed from the short and long arms of the *S. namaycush* Y chromosome hybridize to autosomes in *Oncorhynchus* species, suggesting that the sex chromosomes have, at least in part, evolved independently in the salmonids (Phillips et al., 2001). In chinook salmon, subtractive hybridization has been used to isolate a DNA sequence from the Y chromosome (Devlin et al., 1991). This sequence (*OtY1*) has been used to clone flanking DNA, revealing that it is part of a 8-kb repeat DNA

family present approximately 300 times in the genome (representing a total of 2.4 MB of Y-chromosomal DNA) and organized into at least six clusters of direct-tandem arrays (Devlin et al., 1998). The sequence of this repeat is composed of degenerated retrotransposon sequences (unpublished data), and homologous sequences are observed by PCR and Southern blotting (at much lower copy numbers) in females (Devlin et al., 1991, 1994) and other salmonid species (Devlin et al., 1998), but they are not organized as large arrays of direct-tandem repeats and do not show any sex-specific arrangement. A portion of this sequence has also been independently isolated from chinook salmon using single primer PCR amplification (Clifton and Rodriguez, 1997). The *OtY1* sequence has been used to cytogenetically identify the chinook salmon Y chromosome by in situ hybridization (Stein et al., 2001), and is found located at the distal end of the short arm of an acrocentric chromosome (Fig. 7C). This chromosomal location is consistent with genetic mapping data that found the sex-determination locus in chinook salmon to be located in a chromosomally distal position (Devlin et al., 2001), and in association with a previously characterized variable DAPI-bright band (Phillips et al., 1985). This Y chromosomal sequence is found in many chinook strains examined (Devlin et al., 1991; Clifton and Rodriguez, 1997), although some natural variation has been detected (unpublished observations) and incomplete association of this marker with male development (potentially due to sex reversal effects — see Section 6.4) has also been reported (Nagler et al., 2001). Recently, a repeated retrotransposon sequence (*XIR*-like) has also been discovered on the Y chromosome of *X. maculatus* adjacent to the *Xmrk* locus (Nanda et al., 2000). In situ hybridization studies revealed that the sequence is located at a terminal position on the Y chromosome, but is not present in all strains of *X. maculatus*.

The accumulation and divergence of, and relaxation of selective pressures on, DNA sequence on sex-limited chromosomes is clearly exemplified by the presence of pseudogenes. In mammals, degenerated genes have been identified on the Y chromosome (e.g. Carrozzo et al., 1992; Legouis et al., 1994; Weller et al., 1995), suggesting that mutational damage is not efficiently selected against in these chromosomal regions (see Section 5.3). In salmonids, growth hormone (GH) genes are present in a duplicated form (e.g. Devlin, 1993) on the autosomes arising from the tetraploidization of the genome approximately 30 million years ago (Allendorf and Thorgaard, 1984; McKay et al., 1996). However, in addition to autosomal GH genes, male-specific GH gene sequences have also been identified in chinook, coho, chum and pink salmon (but not sockeye or Atlantic salmon nor rainbow trout) (Du et al., 1993; Forbes et al., 1994; Devlin et al., 2001), and more recently in some Japanese salmon species (Nakayama et al., 1998; Zhang et al., 2001). Analysis of this sex-linked GH sequence reveals it is structurally damaged and is a pseudogene homologous to the autosomal type-2 GH gene (Du et al., 1993). The Y-linked GH sequence segregates independently from the autosomal or pseudoautosomal GH2 locus, suggesting that it arose on the Y chromosome by gene a duplication and/or transposition event (Devlin et al., 2001). That only certain salmonids species contain this Y-linked GH pseudogene implies that the sequence probably arose shortly after the evolutionary origin of salmonids, but was subsequently lost in some lineages. This is also consistent with observations that this nonfunctional pseudogene can be deleted from some individuals in wild salmon populations in Japan (Zhang et al., 2001) and North America (unpublished observations).

5.5. Polyfactorial control of sex determination

Since the pathways involved in sex determination are very complex, it is not surprising to find that many genes are known to influence the process. In many fish species, crosses do not consistently produce 1:1 sex ratios, indicating that Mendelian segregation of sex chromosomes is not responsible for sex determination. In these cases, sex is determined (under constant environmental conditions) by the balance of male and female determining genes, factors that may not be equivalently distributed to offspring. For example, if a male that possessed just sufficient genetic factors to induce male development is crossed with a female with many (or strong) female-determining loci, the resultant progeny will have a high probability of obtaining a preponderance of female-determining factors, and few males may be observed.

5.5.1. Sex ratios from regular crosses

The developmental decision of whether to develop as a male or a female is decided by the relative number and strength of sex-determining genetic factors located on both the sex chromosomes and the autosomes. In species where individual crosses regularly yield sex ratios not differing from 1:1, monofactorial determination of sex with single or tightly linked genetic factors located on a single chromosome is suspected. However, many fish species show deviation from this ratio when sex ratios are examined in individual broods. For example, in *Xiphorphorus*, sex ratios typically range between 0.33 and 0.67 male, the variance arising from various combinations of sex-linked and autosomal sex-determination modifiers (Kallman, 1984a). Similarly, variable sex ratios have recently been reported among separate families in *D. labrax* L. (Gorshkov et al., 1999).

In several model species, exceptions have been identified where the sex-chromosome constitution and phenotypic sex of individuals do not correspond (Yamamoto, 1969). In these cases, autosomal genes capable of affecting sex determination can override the sex-determining activity of the primary sex chromosomes. Such effects have been observed in guppies, medaka, and *Xiphophorus* (see Yamamoto, 1969; Kallman, 1984a,b; Kallman and Bao, 1987). In guppies, wild populations have 1:1 sex ratios (Snelson and Wetherington, 1980), whereas "older" laboratory strains tend to have female-biased sex ratios (Farr, 1981). Rather than being autosomal, this effect is genetically linked to the Y chromosome, and may arise from mate selection pressures, and/or degeneration and reduced viability of the Y chromosome. Breeding experiments with medaka have shown that the frequency of autosomal sex-determination factors can be modified by genetic selection (Aida, 1936), demonstrating that a continuum of genetic sex determination factors exists in some species.

In tilapia, breeding studies indicate that sex is determined by major sex chromosomes (both XY and ZW systems), but that autosomal influences are also operating in many strains (Hammerman and Avtalion, 1979; Wohlfarth and Wedekind, 1991; Trombka and Avtalion, 1993). For example, in *O. niloticus*, an XY system is primarily operating. However, sex ratios are variable and differ significantly among broods, suggesting that polyfactorial influences on sex determination are able to override the sex-determining activity of the major sex chromosomes (Jensen and Shelton, 1979; Scott et al., 1989; Mair et al., 1991b; Tuan et al., 1999b). *O. niloticus* appears to possess a substantial

capability for modification of sex ratio, as heritabilities for sex ratio appear to be quite high ($h^2 = 0.26$; Lester et al., 1989). This plasticity has allowed detection of exceptional individuals where phenotypic sex does not correlate with the constitution of the major sex chromosomes: An XY female that produced YY progeny by gynogenesis has been identified (Scott et al., 1989), and sex ratios in some regular crosses approximated 3M:1F, suggesting that some females may be XY individuals that have followed a female mode of development (Mair et al., 1991b). *O. niloticus* that have a YY genotype produce broods containing a very high proportion of males (average >95%, ranging to 100%), indicating that autosomal influences can be minimized such that sex determination is controlled largely, but not exclusively, by the major sex chromosomes (Scott et al., 1989; Mair et al., 1997; Tuan et al., 1999b; Beardmore et al., 2001). In *O. aureus,* the reciprocal situation has been observed, where a ZW system with autosomal influences was apparent: Some ZW males were identified which produced a 3F:1M sex ratio (Mair et al., 1991a). *O. hornorum* also appears to be controlled by both sex-chromosomal and autosomal influences, as sex ratios among broods range between 47% and 72% males (Obi, 1988).

In common carp, an important discovery has provided evidence for autosomal influences on sex determination, and has revealed how mutations in single genes can cause a complete reversal of the mode of sex determination. During the course of gynogenesis experiments, a recessive autosomal mutation (*mas*) was discovered in common carp that causes XX; *mas*/*mas* individuals to become masculinized and develop an intersex gonad or a testis (Komen et al., 1992b,c). These masculinized XX individuals can develop as functional males, and in crosses with XX; *mas*/+ females, can sire both XX; *mas*/*mas* male and XX; *mas*/+ female progeny (Komen et al., 1992a). Within these strains, the nonmutated + chromosome has essentially been switched from an autosome to a W chromosome, and the original sex chromosomes are no longer involved in the sex-determination process. Although the mutation is usually recessive (Komen et al., 1992a), rare *mas*/+ males can be obtained under unfavourable conditions, leaving the possibility that the mutation may be partially dominant and arose by either gain or a loss of function. A loss-of-function mutation would imply that the normal role of the *mas* gene is to allow female sex differentiation to proceed, and that simply prevention of female development is sufficient to induce male development. Alternatively, a gain-of-function mutation would suggest that male development requires active modification of the sex-determination pathway to encourage testicular rather than ovarian development. Future studies on the nature of the *mas* gene function should be very revealing with regard to sex-determination mechanisms in fish.

5.5.2. Hybridization

The previous section has indicated how male- and female-determining genes are located throughout the genome of many fish species. In some cases, the strength of particular factors overrides all others to allow the development of a single sex chromosome, whereas in other cases, the strengths of all factors are more equivalent and the existence of several minor sex chromosomes may be apparent. In both cases, population genetic forces will tend to create systems where the average sex ratio is 1:1 (Bull, 1983). However, between closely related species, striking differences in sex-factor number,

strength, and location can exist, and when such genomes are placed together in interspecific hybrids, abnormal sex ratios may result.

In tilapia, single-sex populations are desirable to improve productivity in aquaculture. Interspecific tilapia hybrids can produce variable sex ratios, but in some cases, all-male or predominantly all-male progeny result (Avtalion and Hammerman, 1978; Hulata et al., 1983; Majumdar and McAndrew, 1983; Lahav and Lahav, 1990; Wohlfarth and Wedekind, 1991; Mair et al., 1995; Beardmore et al., 2001). For example, in crosses between *O. niloticus* (XX) females and *O. aureus* (ZZ) males, sex ratios vary between 50% and 100% males, whereas *O. urolepis hornorum* males tend to produce a high proportion of males in crosses with other tilapia species. Reciprocal crosses can produce different sex ratios in some cases, indicating that the strengths and chromosomal distributions of male and female determining factors differ between tilapia species.

O. latipes females crossed to *O. curvinotus* males results in all-female progeny (Hamaguchi and Sakaizumi, 1991), whereas in *O. latipes* by *O. celebensis* hybrids, males lack spermatozoa and some fish are missing gonads completely (Iwamatsu et al., 1984). In this latter case, genetic crosses indicate that polygenic female-determining factors in *O. latipes* can override the masculinizing effect of the *O. curvinotus* Y chromosome. Similarly, in salmonid hybrids, sex differentiation and fertility can be impaired (Chevassus, 1983). Some indication that intersexuality, sterility, and single-sex groups arise in hybrids of *Cyprinodon* has also been reported (Cokendolpher, 1980), and in crosses between *P. nigrofasciata* females and *P. caudofasciata* males, all F_1 progeny are females (Yamamoto, 1969).

5.6. Crosses involving sex-reversed individuals

Fish provide excellent experimental systems for analysis of sex determination due to the natural lability of this process and a capacity for functional sex inversion in many species. In many cases, manipulations involving hormone treatments or temperature shocks (see Section 6) are able to modify sexual phenotype independent of the individual's genotype. This capability has allowed investigators to perform crosses between individuals of the same genetic sex, and examination of the ratios and genotypes of the resulting progeny can yield important information regarding the mechanism of sex determination employed by the species in question. Examples of progeny ratios obtained from various species of fish that have been sex reversed are shown in Table 1 for masculinized fish, and Table 2 for feminized animals.

In heterogametic male species, crosses between feminized XY males and regular XY males will yield zygotes with XX, XY and YY genotypes in a 1:2:1 ratio. If the Y chromosome has not degenerated sufficiently to be lethal in a homozygous condition, then the sex ratio of these crosses will be 3:1, males to females, whereas if the Y is homozygous lethal, sex ratios will be 2:1. In practice, it can be very difficult to accurately distinguish sex ratios of either 67% or 75%, and thus it is preferable to test cross progeny males to determine whether they are of XY or YY genotype (producing equal numbers of males and females in the former case, and all males in the latter). Conversely, masculinization of genetic females in an XY system will yield XX males that in crosses to regular XX females will yield all-female progeny. This approach has formed the basis for production of monosex stocks in several aquacultured species (Donaldson, 1996).

Table 1
Sex ratio of progeny from masculinized females crossed to regular females

Species	Sex ratio (%female)	WW survival?	Sex-determination system[a]	References
Betta splendens	8/11 crosses had 100%, 1 had 96%, 1 had 71%, 1 had 55%		XY, and XY + A	(Lowe and Larkin, 1975)[b]
Betta splendens	100%		XY	(Kavumpurath and Pandian, 1994a)
Carassius auratus	100%		XY	(Yamamoto and Kajishima, 1969)
Cichlasoma nigrofasciatum	81%		XY + A	(George and Pandian, 1996)
Clarias lazera	100%		XY	(Liu et al., 1996b)
Cyprinus carpio	100%		XY	(Wu et al., 1990; Komen et al., 1995)
Ctenopharyngodon idella	100%		XY	(Boney et al., 1984; Shelton, 1986)
Dicentrarchus labrax	5–50%		A[c]	(Blázquez et al., 1999)
Gnathopogon caerulescens	70–100%		XY + A	(Fujioka, 1993; Fujioka, 2001)
Oncorhynchus mykiss	100% 96%		XY	(Johnstone et al., 1979b; Okada et al., 1979; Olito and Brock, 1991)
Oncorhynchus tshawytscha	92–100%		XY	(Hunter et al., 1983; Devlin et al., 2001)
Oreochromis aureus	66–76%	yes	ZW + A	(Mair et al., 1991a; Guerrero, 1975)
Oreochromis hornorum	75%		ZW + A	(Obi, 1989)
Oreochromis mossambicus	75–100% 100%		XY + A	(Clemens and Inslee, 1968) (Pandian and Varadaraj, 1990)
Oreochromis niloticus	90–100%		XY + A	(Calhoun and Shelton, 1983; Scott et al., 1989; Mair et al., 1991b; Guilherme, 1992)
Oryzias latipes	F		XY	(Yamamoto, 1958)
Paralichthys olivaceus	90.3% 91.1–100%		XY + A	(Tabata, 1991) (Yamamoto, 1999)
Perca flavescens	100%		XY	(Malison and Garcia Abiado, 1996)
Poecilia reticulata	100%		XY	(Kavumpurath and Pandian, 1993a)
Puntius gonionotus	87.8–100%[d]		XY + A	(Pongthana et al., 1999)
Salmo salar	100%		XY	(Johnstone and Youngson, 1984)
	100%		XY	(Galbreath et al., 1994)
Stizostedion vitreum	100%		XY	(Malison and Garcia Abiado, 1996)

[a] 'A' indicates either an influence from autosomal genetic factors or environmental influences.
[b] Fish were sex-reversed by ovariectomy and gonadal regrowth.
[c] Results differ from karyotypic information in Appendix B.
[d] 1/11 fish examined gave a sex ratio of 1:1.

Table 2
Sex ratio of progeny from feminized males crossed to regular males

Species	Sex ratio (%males)	YY survival	Sex-determination system[a]	References
Betta splendens[b]	100% (triploid)		XY	(Kavumpurath and Pandian, 1992b)
Betta splendens[b]	94–100% (gynogen)	no	XY	(George et al., 1994)
Carassius auratus	75%	yes	XY	(Yamamoto and Kajishima, 1969)
Cichlasoma nigrofasciatum	75–80%	no	XY	(George and Pandian, 1996)
Clarias lazera	75%	yes	XY	(Liu et al., 1996b)
Ictalurus punctatus	67–75%	yes	XY	(Davis et al., 1990)
Oncorhynchus kisutch	75%	yes	XY	(Hunter et al., 1982)
Oncorhynchus mykiss	75%	yes	XY	(Johnstone et al., 1979b)
Oncorhynchus tshawytscha	80%	yes	XY	(Devlin et al., 2001)
Oreochromis aureus	68–100%		ZW + A	(Mair et al., 1991a; Lahav, 1993; Melard et al., 1994; Melard, 1995)
Oreochromis mossambicus	57–82%	yes	XY	(Varadaraj and Pandian, 1989)
Oreochromis niloticus	78–79%	yes	XY + A	(Mair et al., 1991b)
	75%			(Guilherme, 1992)
	6.7–98.6			(Tuan et al., 1999a)
	42.4–54.4[c]			
Oreochromis niloticus (YY × YY)	95.6%	yes	XY + A	(Mair et al., 1997)
Oryzias latipes	67–75%	strain specific	XY	(Yamamoto, 1953, 1955, 1964)
Poecilia reticulata	69–75%	yes, low	XY	(Kavumpurath and Pandian, 1992a)

[a] ‘A’ indicates either an influence from autosomal genetic factors or environmental influences.
[b] By polar body retention.
[c] Crosses between XY females and XX males.

In female heterogametic systems, the same principles apply. Crosses between sex-reversed ZW males and ZW females will yield either 67% or 75% females, depending on the viability of the WW genotype, and crosses between ZZ females and ZZ males produce all-male progeny. In polygenic systems, crosses between sex-reversed females and regular females should theoretically produce a female-biased sex ratio, and crosses between sex-reversed males and regular males would be skewed towards males. However, since it is not possible to know the genotypic sex of individuals in such crosses (which arise from unknown mixtures of autosomal sex factors), sex ratios among individual broods would be 1:1 in some, and in others biased towards the sex opposite to that produced by the reversal treatment. Again, in practice, the segregation of polygenic factors makes such analyses very difficult due to the continuum of sex ratios observed among broods.

In the absence of information about the sex-determining mechanism used in particular fish species, crosses involving sex-reversed individuals can reveal the mode employed depending on the sex ratios obtained. For conclusive determinations, reciprocal crosses involving sex-reversed animals should be performed. Thus, in test crosses with regular animals, if feminized animals produce a mixture of male and female progeny, and individual masculinized fish produce either all-female or mixed broods, male heterogamety is strongly implicated. Conversely, if feminized animals produce broods of either all

males or mixtures of male and female, and masculinized fish produce no monosex broods, female heterogamety is suspected. Obtaining both sexes from both feminized and masculinized animals strongly implies that sex is determined by polygenic or environmental factors, although the ratios of progeny obtained may indicate that major sex chromosomes are playing a significant role.

5.7. Unconventional genetic mechanisms

In most fish species, genetic information is contributed from male and female parents to form diploid offspring. However, contributions can be altered naturally or experimentally such that one parent may provide no genetic information, or may contribute additional entire chromosome sets (Donaldson and Hunter, 1982; Thorgaard, 1983a; Donaldson, 1996). Because haploid fish do not survive in most cases past embryonic or larval stages, only combinations of chromosome sets greater than two have been examined in detail in fish. For example, depending on the contributions made in a particular cross, zygotes can be formed that are: (1) triploid, having two chromosome sets derived from one parent (usually their mother) and one from the other parent, (2) diploid gynogenotes, having two sets from the mother and none from the father, or (3) androgenotes, having no sets from the mother and two from the father. Other combinations are also possible for producing tetraploid, pentaploid, and hexaploid individuals in some species (see below). Chromosome set manipulation techniques provide an opportunity to examine sex-determination mechanism by altering the pattern and balance of gene contribution that occurs in normal crosses.

5.7.1. Induced gynogenesis

Gynogenesis is a mode of reproduction whereby offspring are formed exclusively from maternal genetic information (Thorgaard, 1983a; Solar et al., 1991; Mair, 1993; Donaldson and Devlin, 1996; Arai, 2001). This occurs either naturally by the exclusion of paternal genetic information from the zygote (see below), or experimentally by the destruction of DNA with UV or ionizing radiation. In both cases, the sperm remains functional to fertilize the egg and activate development, but little or no genetic information is contributed to the zygote.

Two types of gynogenesis can occur, involving either meiotic or mitotic chromosomes (Onozato, 1984). In mitotic gynogenesis, the maternal nucleus normally replicates during the first embryonic cell cycle to form two identical haploid chromosome sets. The application of physical shocks at this time (either temperature or pressure are commonly used) can induce these two chromosome sets to remain in the same nucleus and form a diploid cell. Since the two chromosome sets in a mitotic gynogen are derived from a replicated haploid maternal nucleus, these cells are completely homozygous for all loci. Alternatively, meiotic gynogens are formed by shock treatments that induce retention of the second polar body formed after the second meiotic division. The polar body is retained in the egg and fuses with the maternal pronucleus to form a diploid zygotic nucleus. The polar body contains a maternal chromosome set produced during the nonreductional division of meiosis, but is not genetically identical to the haploid egg nucleus due to recombination events that can exchange genetic information among chromosome pairs.

Thus, meiotic gynogens are not homozygous for all loci in their genomes, but do have a higher than normal probability of being isoallelic for loci that map to distal chromosomal regions.

For investigation of sex-determination mechanisms, induced gynogenesis allows examination of the strength and distribution of sex-determination genes, which gave rise to the female phenotype in the mother. For example, in XY or XO systems, female individuals contain only X chromosomes, and meiotic or mitotic gynogenetic progeny derived from them also can contain only X chromosomes and thus develop as females. However, while obtaining all-female progeny from a gynogenetic experiment is consistent with an XX mode of sex determination, it is not conclusive evidence, since special circumstances involving genetic exchange or nonreductional gynogenesis in ZW females may also produce all-female gynogenetic progeny (see below).

If female differentiation is determined by a dominant genetic factor located on the W chromosome, then female individuals are heterozygous (ZW) for sex-determining loci. During mitotic gynogenesis, haploid nuclei either have a Z or a W chromosome, which becomes duplicated after shock treatments. If the W chromosome is not lethal in a homozygous condition, then equal numbers of ZZ males and WW females will result; however, if the W chromosome is not homozygous viable, mitotic gynogenetic progeny from a ZW female will produce only male progeny (assuming the absence of meiotic recombination between the sex-determination locus and the lethal locus).

Meiotic gynogens derived from ZW species can be of several forms. If the sex-determination locus is genetically tightly linked to the centromere (either physically close, or recombination is inhibited in the region), then the reductional division of meiosis will separate the Z and W chromosomes to produce cell types with homozygous chromatids prior to the second meiotic division. In this case, the retention of the second polar body will result in the production of homozygous ZZ or WW zygotes in equal proportions, and again, if WW individuals are not viable, then only male progeny will result. If the sex-determination locus is not genetically tightly linked to the centromere in a ZW system, recombination may cause the exchange of sex-determination loci between centromeres to produce heterozygous ZW chromatids on each chromosome. In this case, sex-determination loci derived from both the Z and W chromosomes may be retained in meiotic gynogens, and in the absence of lethality effects, sex ratios of progeny would be either: (1) essentially 50% female in the case of very low recombination (progeny are all ZZ or WW); (2) between 50% and 100% female if some recombination occurs (67% if random), where progeny comprise equal numbers of ZZ and WW individuals and the remainder ZW; or (3) 100% ZW females in cases where an obligate exchange event with high interference (common in fish) occurs between the centromere and the sex-determination locus. To provide conclusive evidence of sex-determining genotypes in meiotic gynogens, other information, derived from progeny testing or crosses involving sex-reversed individuals to determine the genotype of offspring, may be required.

In the absence of significant autosomal influences on sex determination, sex ratio data in gynogens can be effectively used to genetically map the sex-determination locus relative to the centromere. In the tilapia *O. niloticus*, XY females have been used to map the sex-determination locus by gynogenesis to a position 68.9 map units from the centromere (Mair et al., 1991b). Gynogenesis can also be used to examine and to map genotypes in

hormonally sex-reversed individuals (e.g. catfish; Liu et al., 1996a). Feminized XY individuals can be analyzed in an analogous fashion to ZW females, except that resulting heterozygous progeny will be male (XY) rather than female (ZW). In chinook salmon, gynogenesis has been used to map the sex-determination locus to a distal position on the sex chromosomes (approximately 40 cM) and tightly linked to two Y-linked DNA markers (Devlin et al., 2001).

If sex is not determined by a single genetic locus but is controlled by polygenic autosomal sex-determination loci, then gynogenesis will usually produce female-biased sex ratios in the progeny. The original parental genotype must have been sufficiently biased to have induced female development; however, in most cases these individuals would not be homozygous for female-determining factors. Thus, during meiosis, heterozygous sex-determining loci may segregate alleles to produce gametes that have a sex bias that differs from the original female parent. When such chromosome sets are combined in meiotic gynogenesis, or diploidized in the case of mitotic gynogenesis, zygotes can be formed with sufficient male sex-determining loci to induce testicular development.

Table 3 lists examples where natural and induced gynogenesis have been reported in fish species. A bias towards the production of all-female gynogenetic progeny is apparent, suggesting (but not demonstrating—see above) that in many cases females are the homogametic sex. Where pure XY systems have been demonstrated by other means, essentially all-female progeny are obtained by gynogenesis, (e.g. Refstie et al., 1982). The presence of both male and female gynogenotes obtained in many cases of induced gynogenesis (Table 3) leaves uncertain which type of sex-determination system is operating. However, the presence of both sexes in gynogenetic progeny does allow exclusion with some certainty that a purely female homogametic system is operating, and implicates involvement of either ZW or polyfactorial controls. Test crossing or careful analysis of progeny ratios anticipated from meiotic events can assist in determining whether ZW or polygenic systems are operating (e.g. Van Eenennaam et al., 1999b). Where a mixture of major sex chromosomes and autosomal influences are known to be operating (e.g. *O. niloticus*), both sexes are obtained in meiotic and mitotic gynogenotes but at different ratios from separate female parents, reflecting the distribution of autosomal sex-determining factors among individuals (Müller-Belecke and Hörstgen-Schwark, 1995). Interestingly, in this case, some female parents that produce male and female mitogynes produced only females meiogynes, and male mitogynes crossed to regular females produce sex ratios ranging between all male and all female. These observations are consistent with previous results, indicating a major XY sex-determining system with autosomal genes able to override this system in some individuals (see above).

An unusual result has been obtained involving genotypes of some channel catfish derived from meiotic gynogenesis of a sex-reversed XY individual. In this species, an XY sex-determination system is operating based on crosses involving sex-reversed males and isozyme segregation analysis, and the sex-determination locus is located very close to the centromere (Davis et al., 1990; Liu et al., 1993, 1996a). Consequently, meiotic or mitotic gynogenotes from an XY female should be primarily of two types (XX or YY), yet progeny testing revealed that some males could produce both sexes (Goudie et al., 1995a), indicating unconventional meiosis may occur in XY females, that obligate exchange

Table 3
Sex ratios of meiotic and mitotic gynogenetic progeny

Species	Sex ratio	Sex-determination system[a]	References
Acipenser transmontanus	18–50% M	ZW + A	(Van Eenennaam et al., 1999b)
Barbus barbus	M and F	ZW	(Castelli and Philippart, 1993)
Betta splendens (XX female)	100% F	XY	(Kavumpurath and Pandian, 1994b)
Betta splendens (XY female)	96–100% F	XY	(Kavumpurath and Pandian, 1992b; George et al., 1994)
Carassius auratus	100% F	XY	(Bende, 1982; Zhou et al., 1983)
Carassius auratus	100% F	clonal	(Gomel'skii and Cherfas, 1982; Vasil'yeva, 1990; Gomelsky et al., 1992; Cherfas et al., 1994)
Carassius auratus	all F or all M		(Oshiro, 1987)
Carassius auratus 3*n*	M and F		(Shen et al., 1983)
Clarias gariepinus	100% F	XY[b]	(Galbusera et al., 2000)
Clarias macrocephalus	100% F	XY	(Na-Nakorn, 1995)
Coregonus peled	M and F	A	(Polyakova, 1987)
Ctenopharyngodon idella	100% F	XY	(Stanley et al., 1978; Stanley, 1981; Jensen et al., 1983)
Cyprinus carpio	93–100% F	XY + A	(Nagy et al., 1978; 1981, 1984; Hollebecq et al., 1986; Linhart et al., 1986, 1989; Wu et al., 1986, 1990; Komen et al., 1991, 1992c; Cherfas et al., 1994)
Cyprinus carpio (mas/+ females)	63% F	XY + A	(Komen et al., 1992c)
Danio rerio	100% M	A	(Streisinger et al., 1981; Hoerstgen Schwark, 1993)
Esox masquinongy	67% M	ZW or A	(Dabrowski et al., 2000)
Gnathopogon caerulescens	87.3% F	XY + A	(Fujioka, 1998)
Hypophthalmichthys molitrix	100% F	XY	(Mirza and Shelton, 1988; Xia et al., 1990)
Ictalurus punctatus (XX female)	100% F	XY	(Goudie et al., 1995b)
Ictalurus punctatus (XY female)	M and F, 51.7% M	XY + A?	(Goudie et al., 1995a; Liu et al., 1996a)
Limanda yokohamae	F	XY	(Kakimoto et al., 1994a; Aida and Arai, 1998)
Menidia clarkhubbsi	100% F	clonal	(Echelle et al., 1988)
Misgurnus anguillicaudatus (2*n* and 4*n*)	94–100% F	XY	(Suzuki et al., 1985; Arai et al., 1993; Nomura et al., 1998; Arai, personal communication)
Oncorhynchus kisutch	77.3–100% F	XY	(Refstie et al., 1982; Piferrer et al., 1994a)
Oncorhynchus masou	F	XY	(K. Arai, personal communication; Arai, 2001)
Oncorhynchus mykiss	98–100% F	XY	(Chourrout and Quillet, 1982; Feist et al., 1995; Schmelzing and Gall, 1991)
Oncorhynchus rhodurus	F	XY	(Kobayashi et al., 1994)

(continued on next page)

Table 3 (*continued*)

Species	Sex ratio	Sex-determination system[a]	References
Oreochromis aureus	72.2–94.7% F	ZW + A	(Penman et al., 1987; Avtalion and Don, 1990; Mair et al., 1991a)
Oreochromis mossambicus	100% F	primarily XY	(Penman et al., 1987; Pandian and Varadaraj, 1990)
Oreochromis niloticus	100% F	XY + A	(Shah, 1988)
	95.5% F		(Mair et al., 1991b)
	100% F		(Penman et al., 1987)
	64.7% F		(Müller-Belecke and Hörstgen-Schwark, 1995)
	66.7% F		(Hussain et al., 1998)
Oryzias latipes	100% F	XY	(Naruse et al., 1985)
Pagrus major	F	XY	(Sugama et al., 1989)
Paralichthys olivaceus	97.1–100% F	primarily XY + A	(Tabata, 1991)
			(Kim et al., 1993)
	93–100%[c]		(Liu et al., 1999; Yamamoto, 1999)
Phoxinus eos-neogaeus hybrid	100% F from clonal eggs	clonal	(Goddard et al., 1998)
Poecilia formosa	F	clonal	(Monaco et al., 1984)
	100% F		(Schartl et al., 1995)
Poeciliopsis 2monacha-lucida 3n	100% F	clonal	(Schultz, 1969)
Poeciliopsis monacha-2lucida 3n	100% F	clonal	(Schultz, 1967)
Poeciliopsis monacha-lucida 2n hybrid (hybridogenesis)	100% F	clonal	(Schultz, 1967, 1973)
Polyodon spathula	100% F	XY	(Mims et al., 1997)
Puntius gonionotus	100% F	XY	(Pongthana et al., 1995, 1999)
Rhodeus ocellatus	7 M:1 F	ZW + A	(Kawamura, 1998)
Salmo salar	100% F	XY	(Quillet and Gaignon, 1990)
Silurus glanis	100% F	XY	(Nagy et al., 1978; Bieniarz et al., 1997)
Solea solea	40% F	ZW + A	(Howell et al., 1995)
Takifugu rubripes	93% F	XY + A	(Kakimoto et al., 1994b)
Tinca tinca	100% F	XY	(Linhart et al., 1989)

[a] 'A' indicates either an influence from autosomal genetic factors or environmental influences.
[b] Results differ from karyotypic information in Appendix B.
[c] Sex ratios determined with environmental sex inversion of genetic females stabilized with estradiol treatments insufficient to feminize genetic males.

occurs between the centromere and the sex-determination locus, or that autosomal influences may also play a role in sex determination in some cases (Patino et al., 1996).

5.7.2. Natural gynogenesis

Natural gynogenesis arising from the retention of the second polar body has been detected by the presence of unusual triploids in wild fish populations (Thorgaard and Gall, 1979; Cherfas et al., 1991) and in otherwise lethal hybrid crosses (Seeb et al., 1988). However, several fish species also use gynogenesis as their normal mode of reproduction.

Poecilia formosa is a gynogenetic species (Hubbs and Hubbs, 1932; Schultz, 1971) that forms in nature by hybridization between *P. mexicanna* and *P. latipina* (Monaco et al., 1984). The progeny of *P. formosa* are all females, arising from the production of unisexual diploid ova by apomictic suppression of the first recombination and the first meiotic division (Rasch et al., 1982). Fertilization of *P. formosa* eggs occurs via sperm from related *Poecilia* species (with which *P. formosa* must live sympatrically), but sperm merely activate the eggs and do not contribute genetic information to the zygote. It is possible that such sperm are incompatible with the cytoplasmic environment in the *P. formosa* egg, resulting in paternal chromosome loss as has been described in hybrids of other species (e.g. Ye et al., 1989). Thus, offspring of *P. formosa* are genetically identical to their mothers, including sex-determining loci, and thus the species is propagated in a clonal, all-female fashion.

In the parental species of *P. formosa*, ZW chromosomes have been identified in *P. latipinna* but not *P. mexicanna* (Sola et al., 1990) (Table 1). A female-determining W chromosome is not observed in *P. formosa* (Sola et al., 1992a,b), suggesting that the direction of hybridization involved *P. mexicanna* females, a result consistent with mtDNA analyses (Avise et al., 1991). Hormonally masculinized *P. formosa* possess male characteristics in their somatic tissues, but such fish are functionally sterile (Haskins et al., 1960; Hamaguchi and Egami, 1980). These findings suggest that the female-determining factors in *P. formosa* may be quite strong, and are able to override external influences. Similarly, male sterility ensures that female determination is maintained despite occasional inclusion of paternal DNA in triploids or hybrids, or as subgenomic fragments (Haskins et al., 1960; Schultz, 1971; Schartl et al., 1995; Schlupp et al., 1998).

Certain populations of *Carassius auratus* can also reproduce by gynogenesis (Cherfas, 1966; Cherfas et al., 1994; Dong et al., 1996). For *C. auratus gibelio*, both diploid bisexual and triploid all-female forms have been identified (Zhou et al., 1983; Klinkhardt and Buuk, 1990b; Boron, 1994). Triploid ova are reported to arise by the formation of a tripolar spindle and abortion of meiosis I, followed by a single nonreductional meiotic division (Cherfas, 1966). Egg activation occurs by fertilization with sympatric Cyprinid species, but the rare appearance of *C. auratus gibelio* males in gynogenetic populations implies occasional inclusion of paternal genes (Ding et al., 1992; Rokicki and Kulikowski, 1994). In a strain of *C. auratus gibelio* that is unisexual, hormonal masculinization resulted in males with motile but sterile (aneuploid) sperm (Gomel'skii and Cherfas, 1982), as expected from normal meiosis in triploid testis. Allotetraploids (formed between hybrids of *C. auratus* and *C. auratus gibelio* or *C. carpio*) also can reproduce gynogenetically (Yang et al., 1994), and again, problems with sperm decondensation have been observed. Interestingly, in some populations of gynogenetic *C. auratus*, both male and female progeny can be found, and both have the same chromosome number within strains ($2n = 156$, Shen et al., 1983; $2n = 166$, Chen et al., 1996), albeit different from that found in other triploid carp gynogenetic fish ($2n = 150$, Zhou et al., 1983). For gynogenetic triploid *C. auratus langsdorfi*, formation of triploid ova occurs by suppression of one meiotic division, but recombination still occurs in females, indicating that these gynogenetic fish can reassort sex-determining factors and produce rare male individuals by gynogenetic means (Kobayashi, 1976; Zhang et al., 1992). These examples have revealed how aberrations in chromosome transmission in *C. auratus* can function to create sex-

determination mechanisms that limits the appearance of functional males in populations, although dependence on males from related species for egg activation still remains.

In another Cyprinid species pair, *Phoxinus eos* and *Phoxinus neogaeus*, diploid hybrids are all female and reproduce clonally by gynogenesis (Goddard and Dawley, 1990). Triploid and mosaic forms also exist that contain cells with one or two sets of chromosomes from each parental type, indicating that hybrids produce unreduced diploid ova that can be fertilized with sperm from either parental species. Detailed analysis of meiotic products from *P. eos-neogaeus* diploids reveals that identical and nonidentical diploid ova, as well as haploid ova with only the *P. eos* genome, can be produced (Goddard et al., 1998). Further, in combination with haploid parental sperm, such ova can form all-female gynogenetic females or bisexual diploid groups. In the genus *Cobitis*, diploid, triploid, and tetraploid hybrid forms have been identified (Vasil'eva et al., 1989; Vasil'ev et al., 1990; Vasil'yev et al., 1991), and the triploid form is all female and reproduces gynogenetically by producing unreduced triploid ova. Tetraploids are formed from fertilization of triploid ova with sperm from either *C. granoei* or *C. taenia* males, the former resulting in a gynogenetic all-female form and the latter in a bisexual form.

The previous examples indicate that depending on the balance of sex factors, chromosome compatibilities, and ploidy level, hybrid groups can result that are either only female (in which case reproduction occurs by gynogenesis), or are bisexual with normal gametogenesis. Other examples where hybridization and/or gynogenesis may be operating to produce monosexual populations are in *Menidia clarkhubbsi* (Echelle and Mosier, 1982; Echelle et al., 1988), in *Fundulus heteroclitus* by *F. diaphanus* hybrids (Dawley and Yeakel, 1991), and in the *Rutilus alburnoides* complex (Collares-Pereira, 1985). In the latter hybrids, diploid females transmit both hybrid genomes to diploid eggs, which, when fertilized by a parental species, result in the formation of triploid progeny (Alves et al., 1998). However, such triploids produce predominantly haploid eggs by eliminating the unmatched genome and allowing the homotypic genomes to segregate normally during meiosis to produce haploid eggs. This unique system thus allows exchange of genes between diploids and triploids in the populations, and may explain why this species complex has been so successful. In other cases [e.g. hybrids of *Lepomis gibbosus* and *L. cyanellus* (Dawley et al., 1985), or hybrids between *O. latipes* and *O. curvinotus* (Sakaizumi et al., 1991)], hybrids may form unreduced eggs that result in triploid animals, but these are sterile and thus cannot perpetuate the hybrid condition.

Reproduction by natural gynogenesis provides interesting examples of how meiotic mechanisms can control segregation of sex-determining genes to yield monosexual species. Information regarding the nature of sex-determining factors that operate in these conditions can be difficult to discern since modes of gene segregation cannot be analyzed by standard methods. For all-female species to maintain their sexual determinants, reductional divisions and genetic exchange need to be suppressed, and cytoplasmic incompatibility for sperm must be established to ensure that male sex-determining factors do not enter the clonally propagated genomes (see Section 5.7.3). It is of some interest that no male-only species have been described in fish. Such individuals would need to develop genetic systems that ensure the determination of male sex (i.e. XY or XO conditions) as well as a mechanism to prevent X-chromosome transmission through spermatogenesis. The cost of this strategy to egg-producing individuals in sympatric host species may create

enormous impacts on fitness that select against the development and maintenance of such mechanisms in fish.

5.7.3. Hybridogenesis

In the genus *Poeciliopsis*, natural and artificial hybrid progeny derived from certain species are all females, and these hybrids in turn give rise to unisexual progeny as well (Schultz, 1967, 1973). The most intensely studied form of these hybrids is *P. monacha-lucida,* formed by the hybridization of *P. monacha* females with *P. lucida* males (Schultz, 1973; Quattro et al., 1991). Such hybrids clonally reproduce (Quattro et al., 1992b), but unlike gynogenetic fish, they display biochemical and phenotypic characteristics of both parental species (Schultz, 1969; Quattro et al., 1992a). Reproduction is accomplished by mating with males from the bisexual parental *P. lucida* (or other closely related *Poeciliopsis* species) (Schultz, 1971).

P. monacha-lucida produces ova that are entirely devoid of the original paternal set of chromosomes. During meiosis, a unipolar spindle attaches only to *P. monacha* chromosomes, and *P. lucida* chromosomes are lost and do not contribute to the formation of gametes (Cimino, 1972a). The *P. monacha* haploid ova thus formed are fertilized by *P. lucida* sperm, the chromosomes of which are incorporated into, and expressed within, the developing embryo, reestablishing the *P. monacha-lucida* hybrid unisexual. This process of hybridization and paternal chromosome exclusion has been termed hybridogenesis (Schultz, 1969).

Triploid unisexual forms of *Poeciliopsis* are also known, termed *P. monacha-2lucida* and *P. 2monacha-lucida*, and both reproduce by gynogenesis rather than hybridogenesis. These two forms are believed to originate from the *P. monacha-lucida* hybrid through the rare retention of the paternal genome and production of diploid ova, which are subsequently fertilized with sperm either from *P. monacha* or *P. lucida* (Schultz, 1969; Quattro et al., 1992a). Meiosis occurs differently in the triploid unisexuals than in the diploid hybrid, such that an endoreduplication event occurs in triploids, which results in a hexaploid nucleus that undergoes MI and MII divisions without genetic exchange to produce triploid ova (Cimino, 1972b). Thus, in these gynogenetic triploids, all three parental maternal genomes are retained in the gametes, and fertilizing sperm from *P. lucida* only activate eggs (Schultz, 1967) and do not contribute genetically to offspring.

Other influences regulating sex determination must also be operating to allow formation and maintenance of unisexual hybrids. That diploid *P. monacha-lucida* hybrids are unisexual suggests that female sex-determination genes of *P. monacha* are stronger than male influences carried by the *P. lucida* genome. Indeed, triploid *P. monacha-2lucida* are also female, despite arising from the addition of another *P. lucida* paternal genome. Interestingly, some artificial crosses involving *P. monacha-lucida* have yielded unanticipated sex ratios. For example, in crosses between triploid *P. monacha-2lucida* and males of another poeciliid, *P. latidens*, all-female progeny arise that are identical to the female parent (consistent with its gynogenetic mode of reproduction in triploids). However, in crosses of *P. latidens* males with two different diploid, *P. monacha-lucida* strains (originally termed Cz and Cx; see Schultz, 1969), all-male progeny arise from the Cz strain, whereas Cx produces both males and females. Since both strains produce all-female progeny by hybridogenesis in crosses with *P. lucida*, it appears that the *P. latidens* genome

has a stronger male-determining ability than *P. lucida* when in combination with a *P. monacha* genome. Further, different haploid ova that vary in their sex determination character must be produced from the two diploid hybridogenetic forms.

Poeciliid species represent an extreme in sex-determination mechanisms that utilizes modifications in meiotic processes to ensure transmission of entire female-determining genomes directly from mother to daughter. Although they are very successful (Thibault, 1978), having been clonally propagated in nature for as many as 100,000 generations (Quattro et al., 1992b), these hybridogenetic species remain dependent upon sperm derived from males in sympatric parental species, which creates interesting bispecies sexual selection pressures (Schultz, 1971; Monaco et al., 1984; Vrijenhhoek, 1993).

5.7.4. Androgenesis

Androgenesis is the process by which all nuclear genetic material is derived from the father (Parsons and Thorgaard, 1985; Scheerer et al., 1986; May and Grewe, 1993; Corley Smith et al., 1996). Although this condition can arise naturally in fish (Yamazaki, 1983a; Ye et al., 1989; Blanc et al., 1993), it has also been successfully achieved artificially by fertilizing eggs that have been treated with ionizing or ultraviolet radiation to destroy maternal nuclear DNA [e.g. in trout (Thorgaard, 1992), sturgeon (Grunina and Nejfakh, 1991; Grunina et al., 1995), common carp (Bongers et al., 1994), Japanese koi carps (Rothbard et al., 1999), and tilapias *O. niloticus* and *O. aureus* (Myers et al., 1995; Marengoni and Onoue, 1998)]. Although such zygotes are initially haploid and normally die during embryonic or early larval life, diploidy can be restored by treating the fertilized eggs with a temperature or pressure shock around the time of the first mitotic division. This process effectively doubles the haploid chromosome set, and because individuals are entirely homozygous (Young et al., 1996), the viability of induced androgenotes is usually quite low. Androgenotes have also been produced by fertilizing irradiated eggs with diploid sperm produced either from tetraploid fish (Thorgaard et al., 1990; Blanc et al., 1993) or by chemical fusion prior to fertilization (Araki et al., 1995).

In XY systems such as salmonids or carps, either X- or Y-bearing sperm can fertilize irradiated eggs to yield XX female and YY male androgenotes after shock treatment (Parsons and Thorgaard, 1985; Scheerer et al., 1986; Bongers et al., 1999). The former are regular females, but the males with two Y chromosomes will yield monosex progeny in crosses to regular females, and thus are useful for producing all-male populations of fish. In rainbow trout, evidence has been presented that XX androgenotes can also develop as males (Scheerer et al., 1991), perhaps because of incomplete elimination of maternal DNA that results in an aneuploid conditions that disrupts normal sex determination (see Section 5.7.6).

In zebrafish, methods for inducing androgenesis have been developed, and at least one functional male has been obtained (Corley Smith et al., 1996). As indicated by these authors, further experiments (examining sex ratios and progeny testing) will be revealed to determine whether data from androgenesis correlates with that from gynogenesis, the latter case producing either both sexes (Streisinger et al., 1981) or only males (Hoerstgen Schwark, 1993). Together, the findings from gynogenesis and androgenesis suggest that different strains of zebrafish may have distinct balances of sex determination factors that

function in a polyfactorial fashion (but also see Appendix B for evidence that this species possesses sex chromosomes).

An interesting application of androgenesis has provided information relating to the sex-determination locus in a genetic map for rainbow trout (Young et al., 1998). Clonal XX and YY androgenetic lines were used to generate heterozygous XY F_1 males, which were in turn used for the generation of F_2 diploid androgenetic fish. Because second generation androgenotes contained only individual meiotic products from the F_1 male, analysis of their genotypes with variable DNA markers allowed the rapid generation of a genetic linkage map. While this approach is conceptually similar to gene mapping using gynogenetic haploids (Lie et al., 1994; Postlethwait et al., 1994), it also allows mapping of sex determination loci in XY systems, which has revealed a distal location in the case of rainbow trout (Young et al., 1998).

5.7.5. Polyploidy

Early theories on sex differentiation were influenced by genetic studies with *Drosophila*, where the decision to develop as male or female is determined by the balance between sex-chromosomal and autosomal factors, rather than by the number or type of sex chromosomes present (Bridges, 1925; Bull, 1983). In such cases, diploid individuals with an X chromosome to autosome ratio (X:A) greater than 1.0 develop as phenotypic females (i.e. 2X:2A females and 3X:2A superfemales), whereas individuals with an X:A ratio of 0.5 or less (1X:2A males, with or without the presence of a Y chromosome) develop as males. In this system, changes in autosomal ploidy are also able to influence sex differentiation by altering the X:A ratio. Thus in triploids, 3X:3A animals (X:A = 1.0) are female and 1X:3A individuals (X:A = 0.33) are male, but 2X:3A individuals with an intermediate balance of X chromosomal and autosomal factors (X:A = 0.67) contain mixtures of cells developing as male or female. In systems where sex is determined by genetically dominant activity of genes located on sex chromosomes, ploidy is anticipated to have less of an effect on sex differentiation. However, even dominant systems of control may be affected by dosage effects to some degree since genes involved in mammalian sex determination can act in a dosage-sensitive manner to influence sexual phenotype (Capel, 1998).

Ploidy changes can be manipulated in fish (Donaldson and Hunter, 1982; Thorgaard, 1983a; Donaldson and Devlin, 1996; Benfey, 1999), and in many species, gonadal sex differentiation is disrupted. For example, in salmonids and tilapia, triploid females have poorly developed gonads with very few developing oocytes, whereas spermatogenesis occurs in triploid males, but sterility occurs due to random segregation of trivalents, which produces aneuploid sperm (e.g. Benfey et al., 1986; Hussain et al., 1996; Puckhaber and Horstgen-Schwak, 1996). In triploid females, aneuploid oocytes seem to be impaired from significant development (Nakamura et al., 1987), but since no chromosomes leave the egg until well into oogenesis, the exact mechanism by which gametogenesis is impaired in triploid females remains obscure. The genetic sex of the gonad (presence or absence of the Y chromosome) does not appear to influence sterility, since XXY ovaries and XXX testis show similar characteristics as their diploid counterparts (Krisfalusi and Cloud, 1999). Not all triploid fish are sterile, however (see Section 5.7.3). In carps, both males and females of *C. auratus gibelio* are fertile (Shen et al., 1983), whereas females are fertile and males are sterile in triploid hybrids of *C. carpio* (Wu et al., 1993) (see Section 5.7.2). Natural

examples of fertile triploids have also been reported for *Noemacheilus barbatulus* (Collares-Pereira et al., 1995), and *R. alburnoides* (Collares-Pereira, 1985).

In species where sex is determined by a strong XY system, XXX and XXY triploid individuals are female and male, respectively (Thorgaard and Gall, 1979; Chourrout and Quillet, 1982). In ZW systems, the genetic sex of triploids formed by polar-body retention is difficult to interpret due to the possibility of recombination between the sex-determination locus and the centromere, which could skew sex ratios between 50% and 100% females (see above). In polygenic systems, ploidy changes are anticipated to influence sex ratios due to the differential contribution of genomes from males and females (and their associated concentrations of male- and female-determining factors). In tilapia, sex ratios in triploids of a species with an XY system (*O. niloticus*) were similar to that seen in diploid controls (Penman et al., 1987; Mair et al., 1991b; Puckhaber and Horstgen-Schwak, 1996), whereas a preponderance of males was observed in the ZW species, *O. aureus* (Penman et al., 1987; Mair et al., 1991a). Interestingly, triploid *D. rerio* are all males (Kavumpurath and Pandian, 1990), possibly arising from changes in cell size, which alter regulatory factor concentrations sufficient to shift development towards the male mode. For European sea bass *D. labrax* L., sex ratios were unaffected by the triploid condition (Felip et al., 1999). Interestingly, for the rosy bitterling *Rhodeus ocellatus* (which possesses a ZW system), a single W chromosome in a triploid does not induce female development (Kawamura, 1998) implying that the W chromosome is not fully dominant in the presence of three chromosome sets, or that the sex determination in this species may be operating by measuring the dosage of Z chromosomes (see above).

Higher levels of ploidy have also been observed in fish. Tetraploids have an even number of homologues per chromosome and thus can be fertile, producing diploid gametes. Fertile tetraploid males (XXYY) and females (XXXX) have been produced in rainbow trout by suppressing the first mitotic division after fertilization (Chourrout and Nakayama, 1987; Thorgaard et al., 1990; Blanc et al., 1993). In loach *Misgurnus anguillicaudatus*, triploid, tetraploid, and hexaploid animals have been produced (Arai et al., 1993; Zhang and Arai, 1996; Arai and Mukaino, 1997), and interestingly, male and female progeny are obtained in equal numbers (Arai et al., 1999). Tetraploid hybrid progeny derived from *C. carpio* and *Ctenopharyngodon idellus* can be both functional males and females (Wu et al., 1988), and bisexual diploid and tetraploid *Corydorus aeneus* have been identified in nature (Turner et al., 1992). It is likely that wild tetraploid populations are difficult to establish since their diploid gametes will form sterile triploid individuals in combination with regular haploid gametes from diploids. Thus, tetraploid strains or species may form most readily in small populations where multiple individuals derived from the same aberrant premeiotic event can have a significant probability of interbreeding.

Notwithstanding the difficulties in establishing wild tetraploid populations, polyploidy has naturally occurred several times during the evolution of fish and other vertebrates (Ohno, 1970a,b; Allendorf and Thorgaard, 1984), implying that sex-determination mechanisms must be robust to these changes, at least on an evolutionary scale. In XY systems, initial formation of gametes from heterogametic F_1 tetraploids (XXYY) would be biased towards the production of that sex arising from segregation of paired XX and paired YY chromosomes to produce primarily XY diploid gametes. This has been observed in rainbow trout, where the sex ratio in second generation tetraploids is indeed male biased

(94.5%) (Chourrout et al., 1986). In subsequent generations, tetraploid males would be XXXY, and, assuming divalent pairing of chromosomes occurred during meiosis, would produce essentially 1:1 sex ratios. Any pairing tendency that affected regular segregation of X and Y chromosomes (e.g. trivalent X chromosome segregation, with the Y moving at random) would be met with strong selective pressure to cause homologous chromosomes to rapidly diverge to yield only a single pair of chromosomes involved in sex determination. This process has occurred in salmonids (which are clearly of historical tetraploid ancestry; Allendorf and Thorgaard, 1984), such that some 30 million years after the polyploid event (Devlin, 1993; McKay et al., 1996), only single X and Y chromosomes are apparent in males.

The very large number of studies examining ploidy changes in fish contain little evidence that ploidy-mediated intersexuality occurs. In fish, sex differentiation is controlled by endocrine mechanisms with feedback control systems that regulate signals on organismal as well as cellular levels, which in gonochoristic species, leads either to a male or female differentiated state. Hermaphroditic fish species more resemble the *Drosophila* system of cell-autonomous sex determination, having evolved controls that allow differentiation of both testis and ovary in the same animal while exposed to the same endocrine signals. It would be of interest to examine the effects of ploidy change in hermaphroditic species.

5.7.6. Aneuploidy

Evidence exists that aneuploidy occurs naturally in fish (Chernenko, 1976; Severin, 1979; Zelinskiy, 1985), in hybrids and their progeny (Arefjev, 1989; Ye et al., 1989), and from crosses involving triploid animals with aberrant meiosis (Benfey et al., 1986; Hussain et al., 1996; Benfey, 1999). In such cases, it is anticipated that altered balances of sex determination loci, disrupted development profiles, and altered gene expression in aneuploids (e.g. Devlin et al., 1988) could influence the interpretation of sex determination signals. However, studies clearly identifying aneuploid effects on sex differentiation are lacking in fish. Scheerer et al. (1991) have noted that XX androgenotes of rainbow trout produced from irradiated eggs can develop as males, an effect which could be caused by developmental instability resulting from retention of chromosome fragments derived from the female parent. Similarly, during gynogenesis, irradiation of sperm can induce chromosome fragmentation, which could lead to the production of chromosome fragments affecting sex determination (Disney et al., 1987). In this regard, males have been observed among gynogenetic offspring of *M. anguillicaudatus*, a species where sex is normally determined by an XY system (Nomura et al., 1998), but in this case, temperature effects on sex determination may also be acting (see below).

6. Environmental effects on sex determination

6.1. Exogenous steroids

A very large literature on the manipulation of sex differentiation in fish with exogenous steroids has been comprehensively reviewed (Yamamoto, 1969; Hunter and Donaldson, 1983; Yamazaki, 1983b; Pandian and Sheela, 1995; Nakamura et al., 1998; Piferrer, 2001).

The vast majority of this research has been undertaken to control the reproduction of cultured species, although a few studies have also explored the mechanisms of exogenous steroid action. Experiments have involved different androgens, estrogens, or precursor steroids administered by immersion or in the diet at various stages of development. Different dosages and durations of treatment have also been employed to allow elucidation of the critical periods of sensitivity for sex determination. More than 50 species of fish have been investigated in steroid-mediated sex-reversal studies (Table 4). Summarizing the details of these studies in all species is beyond the scope and goals of the present review, and only an overview of recent information is provided as it relates to the stability of sex determination observed among fish species.

Without knowledge of specific mechanisms, it is difficult to know whether control of steroid biosynthesis occurs early or late in the sex-determination pathway. Although steroid biosynthesis may not represent the initial sex-determination event, it is clear that steroids play a critical role in early differentiation of the gonad into the two sexual types, and probably also for the subsequent maintenance of these conditions. Because steroid synthesis and reception are uncoupled (see Section 4), a point is provided in the sex-differentiation pathway of vertebrates where application of exogenous steroids may influence the course of gonad development. Both estrogen and androgen receptors have been identified in early fish gonads (Fitzpatrick et al., 1994; Chang et al., 1999b), providing a mechanism for the action of exogenous steroids on gonadal differentiation. If sufficient levels of sex steroids are provided to fish, and particularly at stages of development when endogenous pathways have not been fully established, altered sex differentiation may occur (Table 4).

Androgen treatment of fish has in most cases been very effective in inducing masculinization of fish (Hunter and Donaldson, 1983). The modes of administration of these steroids has been by inclusion in the diet or by direct immersion, and less commonly by injection or inclusion in slow-release formulations or silastic implants (Pandian and Sheela, 1995). The most common androgen employed in sex-reversal studies has been 17α, methyltestosterone (MT), being effective in over 25 species examined (Table 4). Similarly, treatment of fish with estrogens (primarily estradiol-17β, ethynylestradiol, and diethylstilbestrol) has in most cases resulted in the feminization of genetic males. Effective oral and immersion dosages of these hormones are approximately 5–500 mg/kg feed and 50–1000 μg/l, respectively, but required values range widely between species, compounds, and treatment regimes. For example, 100% masculinization can be achieved in chinook salmon with only a single immersion in MT at 400 μg/l administered at the time of hatching (Piferrer et al., 1993); treatment of *O. niloticus* required immersion in 5 μg/ml for 75 h followed by dietary treatment at 50 mg/kg for 40 days after first feeding (Lone and Ridha, 1993), whereas complete masculinization of common carp can be accomplished with 100 mg/kg MT in the diet between 40 and 70 days postfertilization (Duda and Linhart, 1992). The potency of different androgens also varies widely, such that the minimum doses causing complete masculinization of *B. splendens* were 8, 15, 60, 90 mg/kg diet for 19-nor-ethynyltestosterone, 17α,-methyltestosterone, 11-ketotestosterone, and androstenedione, respectively (Kavumpurath and Pandian, 1994a). It is presumed that different potencies arise from different affinities for steroid receptors, activities of steroid–receptor complexes, and steroid metabolism.

The timing and duration of treatment is of critical importance for inducing sex inversion in fishes (Piferrer, 2001). In general, the most sensitive period is at a time just prior to, or concomitant with, the initial histological differentiation of the primitive gonad (Hunter and Donaldson, 1983) (see Section 3). Because of vast differences in developmental rates among fishes, the labile stage occurs at different chronological times in different species. For example, in the egg-laying salmonids, this time of sex determination occurs during larval hatching from the egg but can occur much later in other species (e.g. Piferrer and Donaldson, 1989; Kavumpurath and Pandian, 1993b; Blázquez et al., 2001; Piferrer, 2001). Treatments of fish with undifferentiated gonads for as brief a period as 2 h can result in a reversal of sexual differentiation (Piferrer and Donaldson, 1991). In viviparous species, sex determination occurs prior to parturition, thus requiring treatment of the parental female to induce sex change in the offspring (e.g. Takahashi, 1975; Kavumpurath and Pandian, 1993a).

Incomplete sex transformations are regularly observed with steroid treatments in fish, such that development of both testicular and ovarian tissue occurs in the same individual (Table 4). In some cases, the intersex conditions results in the production of functional gametes from hermaphrodites (Jalabert et al., 1975; Chevassus et al., 1988; Liu and Yao, 1995) allowing self fertilization (also achievable with protandrous species using cryopreservation; Happe and Zohar, 1988). However, in gonochorists, it is difficult to know whether intersexuality arises from insufficient treatment (dosage, duration, or timing), or because the particular species is unable to fully respond to exogenous hormone treatment. Among gynogenetic clones of common carp (which are normally all female), the efficacy of masculinization with 17α-methyltestosterone varied among families and was always incomplete under a variety of treatment conditions, suggesting that a significant genetic influence on ability to respond to exogenous androgen exists in this species (Komen et al., 1993). Similarly, rearing temperature has been shown to influence the efficacy of masculinization of gynogenetic carp (Nagy et al., 1981).

Very brief treatments with hormones during early stages of the sex determination period can result in permanent alterations in sexual phenotype, suggesting that a developmental period does exist when permanent switching of the sex determination pathway can occur. However, for some species, the sex ratio of hormone-treated groups has been followed through development, revealing that the sex inversion effect can be transitory (see Section 3.4). For example, groups of rainbow trout fed diets with 17α methyltestosterone (Olito and Brock, 1991) showed complete masculinization soon after treatment, but sex ratios reverted back towards 1:1 when examined 6 months later. Since, for trout, it is known that the labile period for sex differentiation begins much earlier than first-feeding stage, it is possible that individuals had their gonadal pathways partially fixed by the time treatment was initiated by dietary means, and androgen treatment would serve only to temporarily alter the sexual state of gonadal cell differentiation. In studies such as these, care must be exercised to ensure that masculinization has actually occurred initially, rather than simply an impairment or delay of ovarian development, which may produce an undifferentiated or sterile gonad that resembles and immature testis.

Excessive treatments (with androgens or estrogens; Goetz et al., 1979; Sehgal and Saxena, 1997b) can lead to disruptions in gonadal development or sterility (Table 4). These effects may reflect incompatibilities between the exogenous steroids and internal genetic and physiological processes, or pathological effects on gonad development. In this

Table 4
Effects of steroids on gonadal differentiation

Species	T	11KT	MT	DMT	DHT	ET	AND	19N	TRB	MIB	PRO	FM	MTL	E2	EST	AME	17EE	DES	References
Acanthopagrus schlegeli														F					(Chang et al., 1995b)
Acipenser ruthenis														F					(Akhundov and Fedorov, 1994)
Anguilla anguilla			NE														F		(Colombo and Grandi, 1989)
														F					(Degani and Kushnirov, 1992)
			NE														F		(Colombo and Grandi, 1995)
														FH					(Andersen et al., 1996)
Anguilla japonica														F					(Chiba et al., 1993)
Betta splendens			M																(Jessy and Varghese, 1987)
	M																		(Badura and Friedman, 1988)
														F			FH	F	(Kavumpurath and Pandian, 1993b)
		MH	MH			MH	MH												(Kavumpurath and Pandian, 1994a)
Carassius auratus			M												F				(Yamamoto and Kajishima, 1969)
		M																	(Kobayashi et al., 1991)
Carassius auratus gibelio (gynogenetic)			S																(Lou et al., 1994)
Ctenopharyngodon idella			MSH																(Boney et al., 1984)
			M																(Shelton, 1986)
Cyclopterus lumpus														F					(Martin-Robichaud et al., 1994)

Cyprinus carpio		MH										(Nagy et al., 1981)
		MS						FS				(Rao and Rao, 1983)
	M							F				(Nagaraj and Rao, 1987)
		S										(Manzoor Ali and Satyanarayana Rao, 1989)
							S					(Das et al., 1990)
							M					(Das et al., 1991)
		MH										(Komen et al., 1993)
		MS										(Gomelsky et al., 1994)
							M					(Sobhnana and Nandeesha, 1994)
		MS										(Sehgal and Saxena, 1995a)
			S									(Sehgal and Saxena, 1995b)
											F S	(Basavaraja et al., 1997)
								S F				(Sehgal and Saxena, 1997a)
									S F			(Sehgal and Saxena, 1997b)
Danio rerio		M										(Yamazaki, 1976)
Dicentrarchus labrax		MH										(Blázquez et al., 1995)
								HS		FHS		(Blázquez et al., 1998a)
Epinephelus suillus		M										(Kuo et al., 1987)
		M										(Tan-Fermin et al., 1994)
Esox masquinongy		H										(Dabrowski et al., 2000)
Esox lucius					M, H							(Demska-Zakes et al., 2000)
Gambusia sp.		M						F				(Yamamoto, 1969)
Hypophthalmichthys molitrix		MHS										(Mirza and Shelton, 1988)
Ictalurus punctatus				F				F				(Goudie et al., 1983)
			F									(Davis et al., 1992)
						M, NE						(Galvez et al., 1995; Davis et al., 2000)
Limanada yokohamae		M										(Kakimoto et al., 1994a)
Micropterus salmoides		MH						F	F			(Garrett, 1989)
		M										(Porter, 1996)

(continued on next page)

Table 4 (*continued*)

Species	T	11KT	MT	DMT	DHT	ET	AND	19N	TRB	MIB	PRO	FM	MTL	E2	EST	AME	17EE	DES	References
Misgurnus anguillicaudatus															F				(Kubota and Hatakeyama, 1987)
															F				(Kubota et al., 1988)
Mizgurnus mizolepus														FH					(Kim et al., 1997a)
Morone saxatilis			M																(Gomelsky et al., 1999)
Mugil cephalus														F					(Chang et al., 1995c)
Oncorhynchus keta														F					(Nakamura, 1984)
Oncorhynchus kisutch			S H											F H					(Goetz et al., 1979)
			S											F					(Hunter et al., 1982)
			MF		M														(Piferrer and Donaldson, 1991)
														F					(Son, 1991)
														F					(Foyle, 1993)
			SH		S									F					(Piferrer and Donaldson, 1994)
																			(Piferrer et al., 1994b)
Oncorhynchus masou														FH					(Nakamura, 1984)
			M																(Park et al., 1993)
			M																(Nakamura, 1994)
Oncorhynchus mykiss	M																		(Yamamoto, 1969)
															F				(Okada, 1973)
			MSH												HS				(Jalabert et al., 1975)
			S																(Yamazaki, 1976)
			MH											FH					(Johnstone et al., 1978)
			M											F					(Johnstone et al., 1979b)
			M																(Okada et al., 1979)
			M																(Bye and Lincoln, 1981)
			MSH								FH								(Van Den Hurk and Slof, 1981)
			MFS																(Solar and Donaldson, 1985)

				MI									(Cousin-Gerber et al., 1989)
										FH			(Goryczko et al., 1991)
			MH										(Olito and Brock, 1991)
			MH										(Schmelzing and Gall, 1991)
			MHS			MHS							(Feist et al., 1995)
Oncorhynchus rhodurus			M										(Nakamura, 1994)
Oncorhynchus tshawytscha			MS										(Hunter et al., 1983)
			MH										(Baker et al., 1988)
									F			FH	(Piferrer and Donaldson, 1992)
	H	MH	MH	MH									(Piferrer et al., 1993)
										F	NE	NE	(Solar et al., 1994)
Oreochromis aureus			M		M								(Guerrero, 1975)
					M								(Anderson et al., 1978)
												FH *	(Hopkins et al., 1979)
					M								(Shelton et al., 1981)
								MH					(Meriwether and Torrans, 1986)
										F			(Mair et al., 1987)
								M					(Torrans et al., 1988)
												F	(Desprez et al., 1995)
							M						(Galvez et al., 1996)
Oreochromis eurolepus hornorum			M										(Obi, 1989)
Oreochromis mossambicus			M										(Nakamura and Takahashi, 1973)
			MF									F	(Nakamura, 1975)
			M		M								(Guerrero, 1979)
	M												(Nakamura, 1981)
			M										(Das et al., 1987)

(continued on next page)

Table 4 (*continued*)

Species	T	11KT	MT	DMT	DHT	ET	AND	19N	TRB	MIB	PRO	FM	MTL	E2	EST	AME	17EE	DES	References
Oreochromis mossambicus																		F	(Basavaraja et al., 1990)
										M									(Nandeesha et al., 1990)
			M					M											(Varadaraj, 1990)
														NE					(Rosenstein and Hulata, 1992)
										M									(Guerrero and Guerrero, 1993)
			M																(Kuwaye et al., 1993)
																	FH	FH	(Rosenstein and Hulata, 1994)
			MH																(Varadaraj et al., 1994)
Oreochromis niloticus					M	M									F			F	(Tayamen and Shelton, 1978)
			M																(Owusu-Frimpong and Nijjhar, 1981)
			MH																(Nakamura and Iwahashi, 1982)
			M									M							(Phelps et al., 1992)
			M																(Hiott and Phelps, 1993)
			M																(Vera Cruz and Mair, 1994)
			M																(Afonso et al., 1994)
																	F	F	(Potts and Phelps, 1995)
			M										M						(Gale et al., 1995)
			M																(Carvalho and Foresti, 1996)
														F			F	F	(Gilling et al., 1996)
																		F	(Tuan et al., 1999a)

Oreochromis spilurus			M										(Ridha and Lone, 1990)
			M										(Lone and Ridha, 1993)
											F		(Ridha and Lone, 1995)
Oryzias latipes	M		M		M	M	M	M	F	F	F		(Yamamoto, 1969)
			M							F			(Fineman et al., 1975)
Paralichthys olivaceus									F S				(Tanaka, 1988)
			MF						F				(Bang et al., 1995; Kitano et al., 2000)
Perca flavescens			MH						F				(Malison et al., 1986)
Poecilia formosa			S										(Hamaguchi and Egami, 1980)
Poecilia reticulata			M										(Clemens et al., 1966)
		M											(Yamamoto, 1969)
									F				(Takahashi, 1975)
									FH		FH	F	(Kavumpurath and Pandian, 1992a)
				M	MH	MH							(Kavumpurath and Pandian, 1993a)
Poecilia sphenops									FH			FH	(George and Pandian, 1995)
						SM							(George and Pandian, 1998)
Pomoxis nigromaculatus			MH										(Al-ablani and Phelps, 1997)
Pseudocrenilabrus multicolor									FH				(Hackman and Reinboth, 1974)
Puntius gonionotus			MS										(Pongthana et al., 1999)
Salmo salar			MS						F				(Johnstone et al., 1978)
			M										(Johnstone and Youngson, 1984)
									FH				(Sower et al., 1984)
	MH								F				(Herman and Kincaid, 1991)

(continued on next page)

Table 4 (*continued*)

Species	T	11KT	MT	DMT	DHT	ET	AND	19N	TRB	MIB	PRO	FM	MTL	E2	EST	AME	17EE	DES	References
Salvelinus fontinalis			M											FH					(Johnstone et al., 1979a)
Salvelinus namaycush	MH													NE H					(Herman and Kincaid, 1991)
Sebastes schlegeli														F					(Lee et al., 2000)
Sparus aurata														F					(Condeça and Canario, 1995)
Stizostedion lucioperca			MFHS																(Demska-Zakes and Zakes, 1997)
Tilapia zillii			M																(Woiwode, 1977)
			M			M													(Guerrero, 1979)
Tinca tinca			MHS																(Linhart et al., 1995)
Verasper moseri														F					(Mori et al., 1995)
Xiphophorus helleri			M																(Yamamoto, 1969)
			M											F					(Lim et al., 1992)
			M																(Mauricio Nava-Bautista and Rodriguez-Gutierrez, 1997)

M = masculinization; F = feminization; H = hermaphrodite gonads; S = sterilization; NE = no effect. *, in combination with methallibure or cyproterone acetate. Citation of Yamamoto (1969) refers to historical references contained within that review. T, testosterone; 11KT, 11-ketotestosterone; MT, 17α-methyltestosterone; DMT, dimethyltestosterone; DHT, 17α-methyldihydrotestosterone; ET, ethynyltestosterone forms; AND, androstenedione forms; 19N, 19-norethisterone; TBA, trenbolone acetate; MIB, mibolerone; PRO, progesterone; FM, fluoxymesterone; MTL, metalonone; E2, estradiol-17ß; EST, estrone; AME, α-methylene-estradiol; 17-EE, 17α-ethynylestradiol; DES, diethylstilbestrol.

regard, differences in spawning rate noted between regular WZ females and feminized ZZ pseudofemales of *O. aureus* may be due to the expression of the male genotype in the latter (Desprez and Melard, 1998a).

Treatments of fish with excessive androgen can also lead to reduced masculinization, and in some cases induce paradoxical feminization (Nakamura, 1975; Van Den Hurk and Slof, 1981; Goudie et al., 1983; Solar and Donaldson, 1985; Piferrer and Donaldson, 1991; Piferrer et al., 1993; Bang et al., 1995; Demska-Zakes and Zakes, 1997; and references therein). Androgens have been recently shown to interact with the estrogen receptor (Mori et al., 1998), and thus may have direct feminizing effects in some cases. However, androgens are also the immediate precursors to estrogens, and the latter may be elevated if its substrate concentrations are sufficiently high to drive conversion rates through the steroid biosynthesis pathway (via the enzyme aromatase). That androgen aromatization may be responsible for this effect has been tested and confirmed in coho salmon: Paradoxical feminization occurred with aromatizable (17α-methyltestosterone) but not nonaromatizable (17α-methyldihydrotestosterone) androgens (Piferrer and Donaldson, 1991; Piferrer et al., 1993). Interestingly, in channel catfish, essentially 100% feminization occurs with some androgens (Table 4), but in this case, nonaromatizable androgens still retain a feminizing effect (Goudie et al., 1983; Davis et al., 1990), even in the presence of clomiphine citrate, an estrogen–receptor blocker (Davis et al., 1992). Other androgens (trenbolone) do not have this effect, and curiously were able to masculinize channel catfish (Galvez et al., 1995), indicating that only certain androgens can regulate the initiation of female sexual differentiation in channel catfish by an as yet obscure mechanism.

Despite the incomplete effects on sex inversion observed in some cases, experiments involving steroid treatment have very clearly demonstrated that the gonad is capable of stably reversing normal courses of sexual development in a wide range of fish species. Transient treatments of sufficient strength and appropriate timing are usually required to initiate this altered differentiation, and sustained application of the reversing hormone is not needed to maintain the altered sexual state. Further, in the majority of cases, genetic functions that normally act to determine sex do not subsequently rereverse sex differentiation after hormone treatment is withdrawn, suggesting that sex-determination loci primarily act during early development, or that their action is of insufficient magnitude to override the course of sex differentiation established by exogenous steroid treatment. Estradiol treatments have been shown to enhance gonadal aromatase activity in differentiating gonads of grey mullet *Mugil cephalus* and medaka (*O. latipes*) (Chang et al., 1999a; Scholz and Gutzeit, 2000), whereas, in rainbow trout testis, estradiol did not induce aromatase mRNA but other steroidogenic enzyme mRNAs (e.g. 11β-hydroxylase) associated with testicular development were repressed (Govoroun et al., 2001). These data imply that exogenous steroids act, in part, by altering the expression of steroidogenic enzyme genes involved in sex differentiation.

6.2. Temperature and other physical variables

In mammals and birds, embryonic development at the time of sex determination occurs under controlled temperature conditions. However, fish are poikilothermic, and embryonic development proceeds in full exposure to the external physical environment where rela-

tively large temperature alterations can occur. While fish have evolved wide tolerances for such temperature effects to allow development of viable embryos, evidence is accumulating that effects on sex determination may occur. Temperature effects on sex have been now observed in at least eight families of jawed fishes, as well as one Agnathan species.

Sex determination is controlled by the actions of a variety of biochemical pathways involving many different proteins (e.g. transcription factors, steroidogenic enzymes, receptors and second messenger systems, etc.). Since it is well known that temperature can dramatically influence the structure and function of proteins and other macromolecules, temperature fluctuations as are encountered by fish in different habitats could alter sex-determination pathways and influence the probability that development would be male or female. Temperature-dependent sex determination has been extensively studied in reptiles, where exposure to elevated temperature results in female development in some species (Bull and Vogt, 1979; Vogt and Bull, 1982). These temperature-dependent effects appear to be mediated in part by influencing aromatase activity and estradiol synthesis in females, and by steroid receptors in both sexes (Crews and Bergeron, 1994; Crews, 1996). Such effects may also occur in fish: Estradiol secretion has been shown to range as much as 20-fold over just a 5 °C temperature range in common carp (Manning and Kime, 1984), and temperature also affects steroid production testis in trout, carp and tilapia (Kime and Hyder, 1983; Manning and Kime, 1985; Kime and Manning, 1986). In Nile tilapia (*O. niloticus*) and Japanese flounder (*P. olivaceus*), elevated temperatures (which cause masculinization) are associated with reduced aromatase mRNA levels and lower estradiol levels (Kitano et al., 1999; D'Cotta et al., 2001), and treatment with an aromatase inhibitor is able to counter the masculinizing effects of high temperature (Kwon et al., 2000).

In the Atlantic silverside *M. menidia*, incubation of larvae at higher temperatures increases the proportion that differentiate as males (Conover and Kynard, 1981). The temperature-sensitive period was during the mid-larval stage, and subsequent temperature fluxes had no effect on sex ratio, suggesting a switch-type mechanism operates to control sex in this species (Conover and Fleisher, 1986). The temperature responsiveness of *M. menidia* also has a genetic component since progeny from different females respond differently to temperature influences (Conover and Kynard, 1981), and different sires also can have a strong effect on temperature responsiveness (Conover and Heins, 1987a). In nature, ocean temperatures are suspected to affect sex ratio in *Menidia* species such that females are produced from earlier, colder spawning conditions, allowing additional time for ovarian growth (Conover, 1984; Middaugh and Hemmer, 1987). This temperature responsiveness is affected by latitude (Conover and Heins, 1987b), such that northern populations from Canada do not respond to temperature, whereas those from South Carolina do (Lagomarsino and Conover, 1993). These genetic differences allow distinct populations of *M. menidia* to adjust sex ratios appropriately at different latitudes to maximize fitness.

Low temperature is also capable of biasing sex differentiation toward females in two other atherinids, *O. bonariensis* and *Patagonina hatcheri*. The two species show distinctive responses to temperature: *O. bonariensis* sex ratio is influenced without threshold over a broad range of temperatures, whereas *P. hatcheri* sex is only influenced at temperature extremes and otherwise has genetically determined sexes (Strüssmann et al., 1996b,c). The temperature-sensitive period for *O. bonariensis* (Strüssmann et al., 1997) was during the first few weeks posthatching, similar to that seen for *Menidia*.

In the loach *M. anguillicaudatus*, elevated temperature has been shown to skew sex ratios towards male (Nomura et al., 1998). Masculinization was also induced by elevated temperatures in genetically female (gynogenetic) diploids, although interestingly, some males also rarely appeared in certain control crosses as well, suggesting that other factors such as aneuploidy or autosomal sex factors may also influence sex differentiation in this species. In the synchronous hermaphrodite *R. marmoratus*, low temperature incubation (20 vs. 30 °C) increased the proportion of primary males from 3.8% to 74.5%, whereas other physical variables such as salinity and illumination had no consistent effect (Harrington, 1967; Harrington and Crossman, 1976). Recently, the lack of effect of salinity on sex differentiation was confirmed in *O. niloticus* (Abucay et al., 1999).

In a comprehensive survey of temperature and pH effects on sex determination among 39 teleost species, 33 cichlid species in the genus *Apistogramma* (bred from field-collected specimens) and *Poecilia melanogaster* (from a laboratory stock) were found to be significantly affected by larval incubation temperature (Roemer and Beisenherz, 1996). In most but not all cases, increasing temperature (range 23–29 °C) elevated the percentage of males in broods. The effect of pH was less pronounced, but, in general, high pH conditions reduced the proportion of males, in some cases to less than 10% (in *A. caetei*). In contrast, pH has been found to have a significant effect on sex ratio within broods of *Pelvicachromis pulcher*, *P. subocellatus*, *P. taeniatus*, *Apistogramma borelli*, *A. caucatoides*, and *X. helleri*, where low and high pH produce male and female-biased broods, respectively (Rubin, 1985).

Although sex determination in tilapia species is known to be controlled polygenically by major and minor factors on the sex chromosomes and autosomes (see Section 5.5), temperature influences on sex ratio have also been detected (Baroiller and D'Cotta, 2000). In *Oreochromis mosambicus*, genetically female groups (derived from crosses between sex-reversed XX males and regular females) exposed to low temperature (19 °C) incubation during early development resulted in 89% males (Mair et al., 1989). In a similar study, *O. mosambicus* exposed to a range of temperatures (20–32 °C) in early development displayed an increasing proportion of males with elevated temperature (Wang and Tsai, 2000). In *O. aureus* (which has primarily a ZW system), warm temperatures (32 °C) induced differentiation of 20% females compared to 3% observed in controls (Mair et al., 1989), whereas more males (98% vs. 63% in controls) have been observed at higher temperatures in other experiments (Desprez and Melard, 1998b). Fluctuating temperature regimes also can induce masculinization, but less effectively than a constant high (35 °C) temperature (Baras et al., 2000). In *O. niloticus*, elevated temperature generally has a masculinizing effect that is affected by, and can override, genetic influences on sex determination (Baroiller et al., 1995, 1996; Baras et al., 2001), but a feminizing effect has also been observed in all-male and YY strains of *O. niloticus*, particularly in inbred vs. outbred strains (Abucay et al., 1999). Environmental conditions are anticipated to have variable effects on sex differentiation depending on the genetic background and developmental stability of different strains. These observations imply that sex determination is very labile in different tilapia species and that, depending on the exact combinations of genetic modifiers present in different strains, environmental effects on sex determination may be variable in strength and direction, and may also be very sensitive to the level of inbreeding and consequent developmental stability within a strain (Purdom, 1993; Abucay et al., 1999). Temperature lability may provide evolutionary advantages to

tilapia species by providing higher numbers of males with increased capacity for dispersal (Baras et al., 2000), but would also be expected to affect the establishment of sex chromosomes with complete control over the sex determination process and would also result in the accumulation of balancing autosomal genetic factors.

Genetic effects on temperature responsiveness have also been detected in *Poeciliopsis lucida*, a viviparous species: Exposure of embryos from a sensitive strain to elevated rearing temperatures before parturition can bias the sex ratio towards males (Schultz, 1993). Analysis of F_1 progeny derived from reciprocal crosses between responsive and nonresponsive strains also showed that this effect arises primarily from the genotype of the progeny rather than from maternal influences. Recently, elevated temperature has been shown to have a masculinizing effect on sex determination in the honmoroko, *Gnathopogon caerulescens* (Fujioka, 2001), a species with primarily an XX/XY system of sex determination. In this study, intersexes could be sex-reversed. XX males were positively identified by progeny testing, and significant family effects were found to influence sex ratios at normal temperatures as well as the response to masculinizing effects of elevated temperatures.

In *Anguilla*, low-temperature incubation did not affect sex ratio in *A. rostrata* (Peterson et al., 1996), whereas male-biased sex ratios appear to be slightly enhanced by elevated larval incubation temperatures (Holmgren, 1996) in *A. anguilla* or by high stock densities in both species (Roncarati et al., 1997; Krueger and Oliveira, 1999). Based on field studies of lamprey (where gonadal differentiation can change during development; Lowartz and Beamish, 2000), environmental factors may influence sex ratio such that elevated temperatures reduce the incidence of males under high-growth conditions or lower population densities (Beamish, 1993; Docker and Beamish, 1994). In other fish where temperature influences on sex ratio have been specifically examined, no effects have been observed, including northern populations of *F. heteroclitus* and *Cyprinodon variegatus* (Conover and Demond, 1991), the mosquitofish (*G. affinis*) (Bennett and Goodyear, 1978), and the bloater (*Coregonus hoyi*) (Eck and Allen, 1993).

In other fish, indications of temperature-dependent sex determination have been suggested. In channel catfish *Ictalurus punctatus*, sex is normally determined genetically by an XY system, but high temperature extremes applied during the critical period for sex determination result in female-skewed sex ratios, which indicate influence by environmental factors as well (Patino et al., 1996). In sockeye salmon (*Oncorhynchus nerka*), a temperature elevation occurring during embryonic development has been associated with a female-biased sex ratio (Craig et al., 1996), and similarly, elevated temperatures are associated with female-biased sex ratios in *Epiplatys chaperi* (Van Doorn, 1962) and *G. aculeatus* (Lindsey, 1962). At normal rearing temperatures (25 °C), the sex ratio of sea bass (*D. labrax*) populations is normally male biased, but low-temperature incubation (15 °C) during the labile period of gonad development results in all-male populations (Blázquez et al., 1998b; Pavlidis et al., 2000). In contrast, in hirame *P. olivaceus*, high temperature reduced the numbers of females (Tabata, 1995; Yamamoto, 1999), and in the barfin flounder *V. moseri*, a difference in rearing temperature from 14 to 18 °C near the time of gonadal differentiation results in equal sex ratios in the former to all male progeny in the latter (Goto et al., 1999). In marbled sole, *Limanda yokohamae*, masculinization has also been shown to be induced by elevated (25 vs. 15 °C) temperatures (Goto et al., 2000a), as have goldfish and black rockfish *S. schlegeli* (Goto et al., 2000b; Lee et al.,

2000). Some temperature effects on sex determination may be quite subtle (e.g. in atipa *Hoplosternum littorale*), detectable only by careful examination of intrafamily responses that are otherwise masked in populations by genetic variance among different families (Hostache et al., 1995). A discussion of other factors (day length, radiation, water quality, crowding, fertilization timing) reported to influence sex ratio has previously been presented (Chan and Yeung, 1983).

The above studies clearly reveal that sex determination can be influenced by external physical variables such as temperature in most fish families examined. In some cases, the species utilize these influences as a strategy to improve reproductive success, whereas in others, the effects on sex determination may not occur naturally, and may arise from disruptions of normal sex-determination processes under extreme environmental conditions. Indeed, the viability of germ cells of two species of fish (*O. bonariensis* and *P. hatcheri*) have been shown to be sensitive to elevated temperatures (Strüssmann et al., 1998), suggesting that pathological effects on gonadal development may indeed occur. Similarly, temperature has been found to influence expression of a *SRY*-related *Sox* gene in a reptile (Western et al., 1999).

6.3. Behavioural control of sex differentiation

Some Perciforme hermaphroditic fishes can change sex depending on their social interaction with conspecifics (Fishelson, 1970; Robertson, 1972). This fascinating behavioural process has opened new avenues of theoretical considerations on evolutionary forces shaping sex allocation, and has provided an amazing experimental system in which to study the physiology, behaviour and evolution of sex-determination processes (Chan and Yeung, 1983; Warner, 1988; Ross, 1990; Shapiro, 1990; Iwasa, 1991; Francis et al., 1993). The types of hermaphroditic fishes that are female to male sex changers (protogynous) or male to female changers (protandrous) have been previously described in Section 3.2 and Appendix A. The process of sex inversion can involve a complete reorganization of the reproductive system, with replacement of gonadal cell types, duct systems, hormone profiles, and sex-specific behaviours. Changes can begin immediately upon a shift in social status of an individual, and, depending on conditions, can be completed as quickly as a few weeks [e.g. *A. melanopus* (Godwin, 1994b) or as long as several years (e.g. *A. frenatus* (Hattori, 1991)]. The evolution of sex-changing strategies in fish may arise from ontogenetic differences in mortality or growth rate occurring (between the sexes) that are capable of influencing reproductive success (Iwasa, 1991).

Both protogynous and protandrous fish appear to initiate early gonadal development as females. Protandrous fish reverse this early gonadal state in most individuals during initial sexual differentiation to become males, and then reverse it again to an ovarian state during late sex-reversal as members of a breeding social group (Shapiro, 1992). This undifferentiated mode of gonadal development (see Section 3) implies that female gonadal differentiation may be primary in both protogynous and protandrous species. In some species, initial gonadal transformations are not necessarily completed to a state where all individuals possess either male or female gonads: For example, the largest individual within nonbreeders of the protandrous *A. frenatus* can have gonads intermediate between male and female, providing flexibility for the direction of sex change depending on the

course of social dynamics that occurs within the group (Hattori, 1991). Similar flexibility exists among the Gobiidae (see below).

Social units in behavioural sex-changing fish are comprised of a group of smaller individuals of a common sex, and a fewer number (sometimes one) of larger dominant individual(s) of the opposite sex. When a dominant individual exits the social group, or no longer can maintain control of all subordinates, the next largest individual may switch sex to assume the role of the former dominant member. Variations on this strategy are observed both in protogynous species [e.g. *A. squamipinnis* (Fishelson, 1970; Shapiro, 1979, 1986); *Centropyge* sp. (Moyer and Nakazono, 1978b; Moyer and Zaiser, 1984; Sakai and Kohda, 1997); *T. bifasciatum* (Warner and Swearer, 1991); *Epinephelus coioides* (Quinitio et al., 1997); *Trimma okinawae* (Sunobe and Nakazono, 1990); *Xyrichtys pentadactylus* (Nemtzov, 1985); and *Coryphopterus* sp. (Cole and Robertson, 1988; Cole and Shapiro, 1995)] and in protandrous species (e.g. *Amphiprion* sp.; Fricke and Fricke, 1977; Ross, 1978; Fricke, 1979; Ochi, 1989; Godwin, 1994a; Hattori, 1994). The system of dominant animal replacement is not absolute, however, since occasionally high-ranking members of the social group can switch sex even in the presence of a dominant animal (Moyer and Zaiser, 1984).

The mechanisms that fish use to interpret social signals is complex, but is based on the individual's status within the social group and the opportunity that exists to breed with conspecifics (Ross, 1990; Shapiro, 1990). As criteria for sex change, species may utilize behavioural interactions between sexes, size relative to peers, sex ratio of groups, and possibly pheromonal or other chemical stimuli (Shapiro, 1981b; Ross et al., 1983; Cole and Shapiro, 1995). A balance exists between suppression forces (i.e. dominant individuals suppressing sex change through aggression) and inductive forces (presence of smaller individuals of same sex, or size and sex ratio) to assess breeding opportunity within a social group (Ross, 1981; Godwin, 1994a). This balance can shift depending on population density, food resources, and niche availability. Behavioural control systems are not anticipated to be present where strong genetic sex-determination systems are operating (see Section 5).

Since behavioural cues shared among individuals are important for initiating the sex-reversal process, populations tend to be relatively small, or individuals may tend to remain in restricted geographical areas (such as reefs) for extended periods. Since the probability of sex change is influenced by social interactions, population abundance can affect the process in several species. In the protogynous *C. julis*, higher stock densities have been correlated with earlier sex inversion (Lejeune, 1987), but if population densities become very large, sex inversion may also be inhibited, due to increased encounters among dominant individuals (e.g. in protogynous *Centropyge potteri* Lutnesky, 1994), or because individuals may acquire an alternate unpaired mate from the population prior to sex change (Ochi, 1989; Hattori, 1991; Hattori and Yanagisawa, 1991b). Removal of multiple males from larger breeding groups can result in a one-to-one sex inversion of females to replace the lost dominant males (Shapiro, 1980, 1981a), and multiple sex changes can also occur in larger (more than seven animals) but not smaller social groups of *T. duperrey* (Ross et al., 1990). Thus, if population sizes are very small, or no smaller conspecifics are available in the social group, sex change may not occur (Ross et al., 1983; Quinitio et al., 1997) since opportunities for breeding are limited. Some all-female groups of protogynous *A. squamipinnis* may remain stable for many months, yet removal of an individual may destabilize the group and induce sex change in large members (Shapiro, 1979). In another

protogynous species (the redband parrotfish *Sparisoma aurofrenatum*), females must leave the social group to become males, and these individuals then only breed when they gain control of a newly available social group (Clavijo, 1983).

In the Gobiidae, socially controlled, bidirectional sex inversion has been observed. In *Trimma okinawae*, removal of a dominant male from a breeding group can induce sex inversion of the largest female, but such resultant males retain both ovarian and testicular tissue, and can undergo a second sex reversal back to female if another larger male is placed in the group (Sunobe and Nakazono, 1993). In simultaneous hermaphrodite species of *Lythrypnus*, both functional testis and ovary are found within individual fish, yet each only adopts either a male or female behaviour pattern (St. Mary, 1993, 1994, 1996). Such a strategy provides flexibility to rapidly adopt a sex that maximizes reproductive output, depending on species-specific strategies (such as nest building and parental care), or changes in ecology or population structure (sex ratio, relative size, or population and species density).

Social control of sex determination has also been observed in the Midas cichlid *Cichlasoma sitrinellum*. However, in this case, differentiation as male or female is initially determined by an individual's relative size as a juvenile: Animals assessing themselves as larger than average within their group are directed towards male development (Francis and Barlow, 1993). As suggested by the authors, this may reflect a heterochronic variation of the social controls of initial sex determination and subsequent sex inversion described above.

6.4. Pollution

Considerable information exists that indicates environmental pollutants can cause serious impacts on fish reproduction (Kime, 1995, 1998; Sumpter, 1997). The complexity of physiological processes involved in gonadal differentiation and maturation provides many opportunities for chemical interference both in males and females. Disruptions include general effects on gonad morphology, rates of spermatogenesis and oogenesis (including vitellogenesis), ovulation, spermiation, and/or impaired numbers or quality of gametes, as well as effects on reproductive behaviour (e.g. Munkittrick et al., 1992; Jones and Reynolds, 1997). The types of chemicals involved in disruptions include heavy metals, organophosphates, organochlorines, and complex or undefined mixtures of compounds found in pesticides, herbicides, industrial waste, or contaminated waters, soils or sediments from natural environments. In many cases, the biochemical mechanisms by which reproductive impairment is mediated are not known, and impacts are noted by features such as reduced gonadosomatic index or failure to undergo sexual maturity. Specific examples of targeted impacts of chemical pollutants on fish reproduction have also been observed, for example endocrine effects on GnRH-stimulated gonadotropin production, steroid hormone production, steroid-dependent thyroxine and T3 production, and reduced neurological or physiological responses to prostaglandin pheromones (e.g. Leatherland and Sonstegard, 1980; McMaster et al., 1991, 1995; Van Der Kraak et al., 1992a,b; Kime, 1995; Waring and Moore, 1997). In other cases, effects on reproduction may also be caused indirectly by pollution-induced stress, since maturation, sex steroid levels, and gamete quality can all be influenced by stress in teleosts (Campbell et al., 1994; Brooks et al., 1997).

In addition to general impairment of reproduction, some environmental xenobiotics have been noted to induce reversals of sexual phenotype at the biochemical or morpho-

logical levels (Bortone and Davis, 1994; Sumpter, 1997). These effects are not usually complete sex inversions, but rather are associated with induction of sex-specific proteins or the appearance of secondary sex characteristics in the wrong sex, and probably reflect specific action of chemical pollutants interacting with sex-determination and differentiation pathways to reverse the sexual phenotype. For example, exposures of fish to complex mixtures of chemicals in bleached kraft mill effluent (BKME) can result in disruptions in sexual phenotype. In mosquitofish (*G. affinis*), secondary sex characteristics are regulated by androgens (Rosa Molinar et al., 1996), and fish downstream of kraft mill effluent production points can be masculinized, displaying elongation of the anal fin into a gonopodium or effects on female sexual behaviour (Howell et al., 1980; Drysdale and Bortone, 1989; Krotzer, 1990; Cody and Bortone, 1997). This influence, which does not affect gonadal sexual differentiation (Hunsinger et al., 1988), appears to be mediated by androgens derived from microbially degraded plant sterols (i.e. stigmastanol and β-sitosterol) (Denton et al., 1985). In fathead minnows (*Pimephales promelas*), laboratory exposure to BKME also increases the number of fish displaying secondary sex characteristics (Kovacs et al., 1995). In goldfish, such compounds (i.e. β-sitosterol) can decrease in vivo and in vitro production of androgens and reduce gonadal cholesterol levels (MacLatchy et al., 1997), suggesting that this sterol may affect the availability or conversion of cholesterol by *P*450scc to steroids (see Section 4.1). β-sitosterol also has estrogen receptor binding activity in trout and goldfish (Tremblay et al., 1995), and is able to induce vitellogenin in trout in vivo (Mellanen et al., 1996).

Recently, sewage treatment plant effluents have been noted to influence sexual phenotypes of fish, including sex reversal, appearance of intersexual gonads, expression of sex-specific proteins, feminization of male gonadal ducts, or the retardation or advancement of reproductive maturity (Kruzynski et al., 1984; Harries et al., 1996; Lye et al., 1997; Sumpter, 1997; Jobling et al., 1998; Rodgers-Gray et al., 2001; Afonso et al., in press). Notably, some nonionic surfactants present in sewage effluents (e.g. 4-nonylphenol), bisphenol-A, and some phthalates have been shown to have estrogenic activity in fish, being able to bind to estrogen receptors and induce vitellogenin in vitro and in vivo, and impair gonadal development (Jobling and Sumpter, 1993; Ren et al., 1993; Jobling et al., 1995, 1996; Tremblay et al., 1995; Lech et al., 1996; Nimrod and Benson, 1996; Harrelson et al., 1997; Ashfield et al., 1998). In Atlantic salmon, exposure to 4-nonylphenol induces zona radiata eggshell proteins as well as vitellogenin, implying a general estrogenic effect (Arukwe et al., 1997; Madsen et al., 1997). Importantly, some estrogenic endocrine-disrupting compounds can also act as antiandrogens or androgen agonists (Sohoni and Sumpter, 1998), indicating the complexity by which pollutants may exert their effects on sexual phenotypes and function.

In a study with rainbow trout, nonylphenol, chlordecone, PCBs (Aroclor 1245), and lindane were all able to induce vitellogenin and estrogen receptors in trout hepatocytes in vitro (Flouriot et al., 1995). This effect was blocked by some antiestrogens (i.e. tamoxifen), and both nonylphenol and chlordecone can displace estrogen from its receptor, and can induce a reporter gene with an ER element in COS cells (a nonpiscine, nonhepatic cell type), indicating that the feminizing effects of some of these chemicals act directly. In contrast, PCBs and lindane did not affect the reporter gene activity in COS cells, suggesting these compounds have estrogenic activity mediated by metabolites produced

by conversion in hepatic cells. Chlordecone has been shown to bind to the estrogen receptor of the spotted sea trout (Thomas and Smith, 1993), and chlordecone, DDT, and two forms of DDE were able to induce vitellogenin and estrogen receptors in juvenile rainbow trout, despite estrogen-receptor binding affinities between 1000- and 156,000-fold weaker than for estradiol (Donohoe and Curtis, 1996).

Exposure of fish to defined or crude mixtures of organic solutions (e.g. PCBs, oil refinery treatment plant effluent, or harbour dredge material), pesticides, and herbicides also have endocrine disrupting activity in fish, inducing or inhibiting the production of vitellogenin (Chen and Sonstegard, 1984; Chakraborty, 1993; Arukwe et al., 1997; Janssen et al., 1997) or estrogen-responsive reporter gene activities (Zacharewski et al., 1995). Some polyaromatic hydrocarbons (β-napthaflavone and 20-methylcolanthrene) have been shown to influence estradiol (but not testosterone) production from salmon follicles in vitro (Afonso et al., 1997). In this latter case, the mechanism of steroid disruption is not known; however, exposure of ovarian follicles to PAHs has been shown to induce the xenobiotic metabolizing enzyme CYP1A1 (Campbell and Devlin, 1996), an enzyme also implicated in the catabolism of estradiol (Ball et al., 1990; Spink et al., 1992).

Other examples of xenobiotic effects on gonadal sex differentiation in fish have been noted. In the protogynous *M. albus*, acute or chronic exposure of females to malathion reduced in vitro production of sex steroids (both testosterone and estradiol) and aromatase activity, and affected the number of animals with intersex gonads (Singh, 1993). Field observations of sex ratios in sea lamprey populations exposed to the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) indicate a shift from male to female bias (Purvis, 1979), and although indirect effects other than chemical exposure (e.g. population density) may also play a feminization role in this case, TFM has been shown to bind to estrogen receptors and induce vitellogenin in vitro in other fish species (Hewitt et al., 1998). In rainbow trout, ovarian development was impaired and the proportion of females was reduced in laboratory populations exposed to the organochlorine mixture Aroclor 1260 (Matta et al., 1998), whereas no effect was observed for methoxychlor (Krisfalusi et al., 1998). Methoxychlor also was found to have no effect on sex ratios in medaka, whereas, β-hexachlorocyclohexane can induce vitellogenesis and oocyte development in male medaka, resulting in an ovotestis (Wester and Canton, 1986). Nonylphenol or octylphenol has been shown to induce an ovotestis in medaka at both early and late developmental stages (Gray and Metcalfe, 1997; Gray et al., 1999), but effects on sex ratios have not been detected in all studies using this compound (Gray and Metcalfe, 1997; Nimrod and Benson, 1998). Although nonylphenol can masculinize secondary sex characteristics in *Gambussia affinis*, no effect on gonadal sex ratio was observed (Dreze et al., 2000), revealing different sensitivities of primary and secondary sex differentiation to this xenoestrogen. Treatment of adult male medaka with estradiol can transiently induce an ovotestis (Shibata and Hamaguchi, 1988), and ethinyl estradiol injected into medaka eggs results in gonadal feminization of XY medaka with a good correlation between gonadal and secondary sex characteristics was observed providing a useful model for detection of maternally transmitted environmental estrogens (Papoulias et al., 2000). Similarly, injection of DDT into medaka eggs of the same strain results in sex inversion of XY individuals (Edmunds, 1999).

In male common carp, exposure to 4-*tert*-pentylphenol during the critical period for sex determination (24–51 days posthatch) reduced the number of primordial germ cells and

affected gonadal structure, including the induction of oviduct formation (Gimeno et al., 1996, 1997). Treatment during the critical period influenced oviduct morphology depending on the dosage and timing and could induce feminization (Gimeno et al., 1998a), and oviducts were stably retained once formed even if the treatment was withdrawn, providing a useful marker for xenobiotic effects. Treatment of adult males caused demasculinization, impacting spermatogenesis, spermatocrit, and germinal epithelium development (Gimeno et al., 1998b). These studies by Gimeno et al. demonstrate the useful of monosex strains in detecting effects of pollution on sex reversal. This approach, and the use of genetic sex identification using Y-chromosomal DNA markers, has also been used in rainbow trout and chinook salmon. All-male populations of rainbow trout (produced by crosses between YY males and regular XX females) were found not to be affected by nonylphenol (Madigou et al., 2001), whereas XY chinook salmon have been found to develop as females in wild populations in the Columbia River, USA (Nagler et al., 2001), and XY chinook salmon alevin exposed to sewage and pulp mill effluent can be either fully of partially sex reversed (Afonso et al., in press). In these cases, care must be exercised to ensure that the markers are sex specific and very tightly linked to the sex-determination locus in the populations under examination.

During the course of their evolution in aqueous environments, fish have been exposed to a variety of natural endocrine disrupting compounds for which mechanisms have been developed to avoid serious influences on sexual phenotypes. However, the studies outlined above strongly indicate that levels and types of environmental pollutants currently found in aquatic habitats are able to affect sex differentiation and reproductive performance in fish, both at late stages of gonadal function as well as during the early period of gonadal sex determination.

7. Elucidation of sex-determining mechanisms in fish species

To elucidate the mode of sex determination used in a particular species, a defined set of experiments should be carried out which investigates the influence of environmental factors, the role of genetic factors, and the stability of sex determination under conditions known to affect the process in other species.

7.1. Cytogenetic studies

One of the most rapid ways that the sex-determination mechanism used by a species can be discerned is by determining the karyotype of male and female individuals. Although many species do not display heteromorphic sex chromosomes (see Appendix B), if they do, the mode of sex determination can be defined with certainty as genetic (see Sections 5.2 and 5.3).

7.2. Analysis of sex ratios among families

Sex-determination mechanisms can also be determined from sex ratios among families. Progeny derived from 20 to 50 single-pair crosses should be produced under controlled

conditions, and sex ratios determined where mortality is shown not to be a significant influencing factor. If sex ratios do not deviate significantly from 1:1 among families, it is very likely, but not certain, that sex is determined by a chromosomal sex-determination system (see Section 5.5). Sex ratios should be examined at several life history stages and different age classes (i.e. pre- and post-first maturity) to investigate the possibility of sequential hermaphroditism. Sex ratios differing from 1:1 may implicate polyfactorial or environmental controls (see Sections 5.5.1 and 6), in which case it may be desirable to perform more complex breeding experiments involving multiple crosses per parent to determine the distribution of genetic factors among individuals, or to conduct experiments where physical variables are altered (e.g. temperature, pH, rearing density, growth rate, etc.) to examine external influences.

7.3. Examine progeny sex ratios from sex-reversed individuals

If experiments examining sex ratios among families do not produce relatively equal numbers of males and females, then experiments described in this section may not provide significant further data. However, if male/female ratios are consistent under a variety of conditions and in many different crosses, this is most likely produced by either an XY or ZW sex-determination system, and environmental and polygenic factors are probably minor influences. To distinguish between XY and ZW modes, sex-reversed individuals should be produced using estrogen and androgen treatments (see Section 6.1), and crosses performed with regular sex fish. As described in Section 5.6, progeny sex ratios can determine which sex is heterogametic (the sex-reversed parent yielding a 3:1 sex ratio biased to the opposite sex is heterogametic), and crosses involving both types of sex-reversed individuals should be used. In practice, the production of functional sex-reversed animals may provide some experimental challenge since the dose and timing of treatment may need to be determined empirically for each species (see Section 6.1), but in the majority of cases, fertile sex-reversed individuals can be produced. A variety of different steroids should be employed, starting with estradiol-17β and ethynylestradiol for feminizations, and 17α-methyltestosterone and the nonaromatizable 17α-methyldihydrotestosterone for masculinizations (see Table 4).

7.4. Development of monosex strains

In the absence of molecular markers, an alternative strategy for genetic sex identification involves the production of genetically defined all-female or all-male strains (Donaldson and Devlin, 1996; Beardmore et al., 2001). Monosex strains can be produced by combining a defined set of genetic crosses with sex-reversal technology, and are simplest to implement when a chromosomal sex-determination systems is operating, although theoretically, it may also be possible to develop monosex strains in polygenically controlled systems by selective breeding.

Monosex stocks can be produced by continuing the experiments described in Section 7.3 above. For example, in XY systems, masculinized XX fish develop as functional males and will produce all-female progeny in crosses with regular females (see Section 5.6). Such lines can be maintained by treating a portion of the progeny with androgen at the time of

sex determination to allow production of sex-reversed XX broodstock males for the next generation (a procedure routinely employed by the aquaculture industry). However, since regular males are also present in first-generation androgen-treated groups, initial establishment of monosex fish requires distinguishing between masculinized XX males and regular XY males. This can be accomplished by test crossing each individual male (with regular females) to identify XX fish that produce only female progeny. Since the labile period for sex determination occurs prior to morphological or histological differentiation of the gonad (needed to evaluate the test cross), the establishment of separate androgen-treated groups for each test cross must be performed in the first generation.

Alternative methods can be used to identify the genetic sex of sex-reversed individuals. For example, gynogenetic offspring from species using an XY system will necessarily be XX fish, thus ensuring that all sex-reversed males developing from androgen-treated groups have a female genotype. Alternatively, hormone treatments may be applied at doses or times that induce incomplete sex inversion, resulting in hermaphroditic animals (see Section 6). Thus, with an XY species, androgen treatments that produce animals with an ovotestis are usually of XX genotype, and sperm isolated from testis tissues (by gentle homogenization) can be used to establish monosex strains. In a similar strategy, application of hormones at late stages of sex determination can result in incomplete sex inversions, producing androgen-treated XX animals with a testis that lack a sperm duct (Johnstone et al., 1979a,b; Johnstone and Youngson, 1984; Bieniarz et al., 1991).

For the production of male monosex strains in an XY system, two approaches may be used. The first is similar to the procedure used to produce all-female strains, but requires one additional generation to accomplish. In crosses involving feminized animals described above in Sections 7.1–7.3, XY females can be identified by test crosses with regular XY males, and if the Y chromosome is homozygous viable in the species (see Sections 5.3 and 5.6), these test crosses should produce 25% YY males. If estrogens are again applied early in development to a portion of the test-cross progeny, YY female fish can be produced. YY females can be distinguished from XY females by test crossing to male siblings from untreated groups (which will either be XY or YY males). If sufficient crosses are performed, some will be YY female by YY male, and estrogen treatment again applied to a subset of their progeny will allow establishment of YY female broodstock. This protocol can be facilitated by cryopreservation of sperm from YY males identified in earlier generations. An alternative method for producing all-male stocks involves the use of androgenesis (see Section 5.7.4): In XY species, approximately 50% of the progeny obtained will be of YY genotype, and if feminized with estrogens, such animals can be used in crosses with androgenetic YY male siblings to produce all-male strains that can be propagated by sex inversion of a portion of the progeny produced in each generation.

For ZW species, crosses involving sex-reversed and regular fish can also be used to develop monosex strains in an approach complementary to that described above for XY systems.

7.5. Isolation of sex-specific DNA markers

Isolation of sex-linked genetic markers has been accomplished for several fish species (see Section 5.4). The haploid genome size of typical fish species is approximately

1.0×10^9 bp, distributed among approximately 25 chromosomes (Hinegardner and Rosen, 1972). If chromosome arms are assumed to have a recombinational length of 50 map units (as is found in many other organisms), and number 1.5 times the number of chromosomes (assuming equal numbers of acrocentric and metacentric chromosomes), typical total genetic lengths of fish genomes are expected to be approximately 1875 map units. For sex-linked markers to be useful in diagnostic assays for genetic sex, they need to be exceedingly closely linked to the sex-determination locus, on the order of less than 0.1 map unit away. With approximately 1Mb of DNA per map unit, it is clear that tens of thousands (1.0×10^9 bp/(10^5 bp/marker)) of randomly selected clones would need to be examined to isolate a useful genetic marker if the sex-determination locus is the only sex-limited gene on the sex chromosomes. Some sex-linked DNA markers have been identified by chance in fish (Du et al., 1993; Prodohl et al., 1994), but fortunately, two factors can improve the probability of recovering sex-specific clones. The first factor is that many sex chromosomes have significant proportions of their length developed as cytogenetically distinct structures (see Sections 5.3 and 5.4.3), thus making the target for isolating sex-specific DNA much larger than the size of a single gene. For example, sex-specific DNA isolated from the Y chromosome of chinook salmon comprises over 2 Mb of sequence on this chromosome (Devlin et al., 1998).

The second factor enhancing recovery of sex-specific DNA is that molecular biology methods have been developed that improve recovery of sequences that are present in one genome and absent in another. In one suite of such techniques, many DNA fragments can be examined simultaneously, thus allowing the genome to be surveyed much more rapidly than by using randomly selected single markers. Techniques in this class use a comparison of fragment patterns obtained between males and females, and include DNA fingerprinting using multilocus minisatellite DNA probes (Wright, 1993), Rapid Amplification of Polymorphic DNA (RAPD) (Iturra et al., 1998), and amplified fragment length polymorphisms (AFLPs) (Young et al., 1998). In cases where polymorphism may be anticipated at a marker locus, analyses involving pooled DNA from several siblings of the same sex (bulk segregant analysis) may be beneficial (Williams et al., 1993). However, these methods only improve the efficiency of screening for sex-specific markers by approximately 1 to 2 orders of magnitude, and still leave a daunting task in many cases.

Another general approach involves subtraction of sequences between one genome and another to allow selective isolation of sex-specific sequences. The basis of these approaches is that the homogametic sex contains all sequences that the heterogametic sex does, except for those in the vicinity of the sex-determination locus. Thus, in XY systems, female DNA can be "subtracted" from the male genome, to leave only male-specific DNA sequences. Such subtraction methods include hybridization approaches (e.g. the Phenol-Enhanced Reassociation Technique (PERT); Kohne et al., 1977; Devlin et al., 1991) or PCR amplification (e.g. Representational Difference Analysis, RDA; Lisitsyn et al., 1993), and can improve the efficiency of identifying unique sequences many thousand fold, making feasible experiments searching for sex-limited DNA sequences. In cases where the mode of sex determination is known, subtraction procedures can be performed in one direction only (i.e. in XY systems, subtraction of male against female genomic DNA), but where this information is lacking subtraction must be performed bidirectionally.

Once putative sex-specific DNA sequences have been isolated, they should be analyzed in defined families to ensure cosegregation with the sex-determination locus. In most cases, it will also be desirable to characterize the clones in detail to allow development of simple PCR diagnostics, to improve testing procedures (improve speed, and allow determination of genetic sex from minute, nonlethal samples of tissue or blood) and to assist in the detection of exceptional offspring (Du et al., 1993; Devlin et al., 1994; Matsuda et al., 1997; Iturra et al., 1998; Coughlan et al., 1999). The necessity of such characterization is exemplified by a study involving a Y-linked DNA marker for medaka, which is sex-linked only in some strains of this species (Matsuda et al., 1997).

8. Conclusion and future

A summary of sex-determination processes in fish (Fig. 1) reveals a complex pattern of variables and mechanisms utilized among different fish orders. Many mechanisms are shared among groups of fish orders, and some orders (e.g. Perciformes) employ many different approaches for controlling sex differentiation. This rich species array of biological material provides a significant opportunity for future sex determination research, promising to yield much new exciting information about how fish control gonadal differentiation at the cellular, organismal, population, and species levels. Emerging genomics technologies capable of analyzing the genetic and physiological constitutions of organisms in great detail are now allowing identification of many of the major molecular components involved in cellular gonad differentiation, in both germ and somatic cell lineages. These tools and approaches enable examination of how developmental cascades of gene expression, physiology, and metabolism unfold in the developing embryo, how they can be affected by environmental variables, and how they can be reprogrammed naturally in hermaphroditic species and can be affected abnormally in exceptional or experimental individuals. Many components identified as potential effectors of sex-differentiation in fish will be able to be tested, using transgenesis or by genetic mapping of natural and induced allelic variants, to examine their necessity and function. Understanding the genetic and physiological components of sex-determination systems will also allow us to specifically test hypotheses relating to the evolutionary pressures that shape sex-determination pathways. Comparative study of the abundant species and sex-determination systems existing in fish is likely to reveal a fascinating competition of alleles within and among genetic loci involved in sex determination, each *selfishly striving* for stronger influence over pathways without disrupting fitness of the individual. Understanding how and why such differences have emerged, and how they are maintained and altered by different selection regimes in nature, will provide a significant and rewarding challenge for the future.

Acknowledgements

Sincere gratitude is extended to E. Jinghan and J. Khattra for assistance in the preparation of Appendices A and B, and to T. Kobayashi, M. Matsuda, C.E. Morrey, M.

Nakamura, R. Phillips, J. Stein, and M. Tanaka for providing figures and unpublished information. The authors also appreciate the very useful scientific and editorial comments provided by and T. Hutchinson, P. Campbell, E. Donaldson, E. Jinghan, M. Matsuda, J. Smith, D.S. Wang, and three anonymous reviewers who spent a great deal of time reviewing the manuscript, and provided many useful suggestions. RHD gratefully acknowledges the Endocrine Modulators Steering Group of the European Chemical Council who provided initial support for the preparation of this review, and YT gratefully acknowledges funding from CREST of JST (Japan Science and Technology).

Appendix A. Hermaphroditism in fish

Order	Family	Species	Type	References
Myxiniformes	Myxinidae	*Eptatretus stouti*	H	(Gorbman, 1990)
Aulopiformes	Chlorophthalmidae	*Chlorophthalmus albatrossis*	S	(Smith, 1975; Ota et al., 2000)
Anguilliformes	Muraenidae	*Echidna nebulosa*	G	(Fishelson, 1992)
		Gymnomuraena zebra	G	(Fishelson, 1992)
		Gymnothorax fimbriata	G	(Fishelson, 1992)
		Gymnothorax flavimarginatus	G	(Fishelson, 1992)
		Gymnothorax gracilicauda	G	(Fishelson, 1992)
		Gymnothorax margaritophorus	G	(Fishelson, 1992)
		Muraena pavonina	G	(Fishelson, 1992)
		Rhinomuraena spp.	A	(Fishelson, 1990, 1992)
		Uropterygius fasciolatus	G	(Fishelson, 1992)
		Uropterygius polyspilus	G	(Fishelson, 1992)
		Siderea grisea	S	(Fishelson, 1992)
		Siderea picta	S	(Fishelson, 1992)
		Siderea thyrsoidea (*Siderea thyrzoidea*)	S	(Fishelson, 1992)
Clupeiformes	Clupeidae	*Tenualosa toli*	A	(Milton et al., 1997)
Cypriniformes	Cobitidae	*Cobitis taenia bilineata* (*canestrini*)	A	(Lodi, 1980)
	Cyprinidae	*Barbus tetrazona tetrazona*	G	(Takahashi and Shimizu, 1983)
Cyprinodontiformes	Cyprinodontidae	*Rivulus marmoratus*	S	(Koenig et al., 1982; Soto et al., 1992; Soto and Noakes, 1994; Lubinski et al., 1995)
	Poeciliidae	*Xiphophorus helleri*	G	(Lodi, 1979)
Perciformes	Callanthiidae	*Callanthias parini*	G	(Anderson and Johnson, 1984)
	Centracanthidae	*Spicara maena*	G	(Reinboth, 1979)
		Spicara smaris	G	(Tsangridis and Filippousis, 1992)
	Centropomidae	*Lates calcarifer*	A	(Moore, 1979; Guiguen et al., 1993, 1994, 1995)
	Cichlidae	*Amphilophus citrinellus* (*Cichlasoma citrinellum*)	H?	(Francis and Barlow, 1993)
		Crenicara punctulata	G	(Carruth, 2000)
	Cirrhitidae	*Amblycirrhitus pinos*	G?	(Sadovy and Donaldson, 1995)

Order	Family	Species	Type	References
		Cirrhitichthys aprinus	G?	(Kobayashi and Suzuki, 1992; Sadovy and Donaldson, 1995)
		Cirrhitichthys aureus	GA	(Kobayashi and Suzuki, 1992)
		Cirrhitichthys falco	G?	(Kobayashi and Suzuki, 1992; Sadovy and Donaldson, 1995)
		Cirrhitichthys oxycephalus	G?	(Sadovy and Donaldson, 1995)
		Cirrhitus pinnulatus	G?	(Sadovy and Donaldson, 1995)
		Cirrhitops hubbardi	H	(Kobayashi and Suzuki, 1992)
		Cyprinocirrhites polyactis	H	(Kobayashi and Suzuki, 1992)
		Neocirrhites armatus	G	(Sadovy and Donaldson, 1995)
	Gobiidae	*Coryphopterus alloides*	G	(Cole and Shapiro, 1990)
		Coryphopterus dicrus	G	(Cole and Shapiro, 1990)
		Coryphopterus eidolon	G	(Cole and Shapiro, 1990)
		Coryphopterus glaucofraenum	G	(Cole and Shapiro, 1992, 1995; Cole et al., 1994)
		Coryphopterus hyalinus	G	(Cole and Shapiro, 1990)
		Coryphopterus lipernes	G	(Cole and Shapiro, 1990)
		Coryphopterus nicholsii	G	(Breitburg, 1987; Kroon and Liley, 2000)
		Coryphopterus personatus	G	(Robertson and Justines, 1982)
		Coryphopterus thrix	G	(Cole and Shapiro, 1990)
		Coryphopterus urospilus	G	(Cole and Shapiro, 1990)
		Eviota afelei	G	(Cole, 1990)
		Eviota disrupta	G	(Cole, 1990)
		Eviota epiphanes	G	(Cole, 1990)
		Eviota fasciola	G	(Cole, 1990)
		Fusigobius neophytes	G	(Cole, 1990)
		Gobiodon micropus	GA	(Nakashima et al., 1996)
		Gobiodon oculolineatus	GA	(Nakashima et al., 1996)
		Gobiodon quinquestrigatus	GA	(Cole, 1990; Nakashima et al., 1996)
		Gobiodon rivulatus rivulatus	GA	(Nakashima et al., 1996)
		Gobiosoma multifasciatum	G	(Robertson and Justines, 1982)
		Lophogobius cyprinoides	G	(Cole, 1990)
		Lythrypnus dalli	S	(St. Mary, 1993, 1994)
		Lythrypnus zebra	S	(St. Mary, 1993, 1996)
		Paragobiodon echinocephalus	GA	(Cole, 1990; Kuwamura et al., 1994; Nakashima et al., 1995)
		Priolepis hiplotti	GS?	(Cole, 1990)
		Priolepis eugenius	G	(Cole, 1990)
		Trimma okinawae	GA	(Sunobe and Nakazono, 1990, 1993)
		Trimma caesiura	GS?	(Cole, 1990)
		Trimma taylori	GS?	(Cole, 1990)
		Trimma unisquamis	GS?	(Cole, 1990)
	Grammatidae	*Gramma loreto*	G	(Amador, 1982; Asoh and Shapiro, 1997)

Order	Family	Species	Type	References
	Labridae	*Achoerodus viridis*	G	(Gillanders, 1995)
		Anampses geographicus	G	(Leem et al., 1998)
		Bodianus diplotaenia	G	(Hoffman, 1983)
		Bodianus eclancheri	G	(Hoffman, 1983)
		Bodianus rufus	G	(Hoffman, 1983)
		Choerodon azurio	G	(Nakazono and Kusen, 1991)
		Choerodon schoenleinii	G	(Ebisawa et al., 1995)
		Cirrhilabrus temminckii	G	(Kobayashi and Suzuki, 1990)
		Clepticus parrae	G	(Warner and Robertson, 1978)
		Coris julis	G	(Reinboth, 1979; Bentivegna et al., 1985; Buslé, 1987; Lejeune, 1987; Reinboth and Bruslé Sicard, 1997)
		Epibulus insidiator	G	(Leem et al., 1998)
		Halichoeres bivittatus	G	(Laming and Ebbesson, 1984)
		Halichoeres garnoti	G	(Warner and Robertson, 1978)
		Halichoeres maculipinna	G	(Warner and Robertson, 1978)
		Halichoeres marginatus	G	(Shibuno et al., 1993a,b, 1995)
		Halichoeres melanochir	G	(Yogo, 1985)
		Halichoeres pictus	G	(Warner and Robertson, 1978)
		Halichoeres poeyi	G	(Warner and Robertson, 1978)
		Halichoeres poecilopterus	G	(Fukui et al., 1991; Lee et al., 1991; Fujiwara et al., 1992)
		Halichoeres radiatus	G	(Warner and Robertson, 1978)
		Halichoeres scapularis	G	(Leem et al., 1998)
		Hologymnosus annulatus	G	(Leem et al., 1998)
		Labroides dimidiatus	G	(Kuwamura, 1984)
		Labrus bergylta	G	(Dipper and Pullin, 1979)
		Labrus bimaculatus (*Labrus ossifagus*)	G	(Dipper and Pullin, 1979)
		Nelabrichthys ornatus	G	(Andrew et al., 1996)
		Notolabrus celidotus (*Pseudolabrus celidotus*)	G	(Jones, 1980a)
		Pseudolabrus japonicus	G	(Lee et al., 1992b; Morita et al., 1997)
		Pteragogus flagellifer (*Pteragogus flagellifera*)	G	(Lee et al., 1992a)
		Semicossyphus pulcher	G	(Cowen, 1990)
		Stethojulis trilineata	G	(Leem et al., 1998)
		Symphodus melanocercus	G	(Warner and Lejeune, 1985)
		Symphodus ocellatus	G?	(Warner and Lejeune, 1985; Bentivegna, 1989)
		Symphodus roissali	G	(Warner and Lejeune, 1985)
		Symphodus tinca	G	(Warner and Lejeune, 1985)
		Thalassoma bifasciatum	G	(Warner and Robertson, 1978; Warner and Hoffman, 1980; Kramer et al., 1988; Koulish and Kramer, 1989; Warner and Swearer, 1991; Kramer et al., 1993; Shapiro and Rasotto, 1993)

Order	Family	Species	Type	References
		Thalassoma cupido	G	(Meyer, 1977)
		Thalassoma duperrey	G	(Ross, 1981, 1987; Ross et al., 1983, 1990; Nakamura et al., 1989; Hourigan et al., 1991)
		Thalassoma lucasanum	G	(Warner, 1982)
		Thalassoma lutescens	G	(Shibuno et al., 1994)
		Thalassoma pavo	G	(Wernerus and Tessari, 1991)
		Thalassoma purpureum	G	(Leem et al., 1998)
		Thalassoma quinquevittatum	G	(Leem et al., 1998)
		Xyrichtys dea	G	(Leem et al., 1998)
		Xyrichtys geisha	G	(Leem et al., 1998)
		Xyrichtys martinicensis	G	(Clark et al., 1988)
		Xyrichtys melanopus	G	(Clark and Shen, 1986)
		Xyrichtys novacula (*Xyrichthys novacula*)	G	(Bentivegna and Rasotto, 1987)
		Xyrichtys pavo	G	(Clark and Shen, 1986)
		Xyrichtys pentadactylus	G	(Nemtzov, 1985; Clark and Shen, 1986; Clark et al., 1988)
	Lethrinidae	*Lethrinus lentjan* (Lacepede 1802)	G	(Wassef, 1991)
		Lethrinus mahsena (Forsskal 1775)	G	(Wassef, 1991)
		Lethrinus miniatus	G	(Church, 1997)
		Lethrinus rubrioperculatus	G	(Vidalis and Tsimenidis, 1997)
	Malacanthidae	*Branchiostegus japonicus*	H	(Watanabe and Suzuki, 1996)
		Lopholatilus chamaeleonticeps (*Lopholatilus chameleonticeps*)	G?	(Erickson and Grossman, 1986)
	Moronidae	*Morone saxatilis*	A?	(Moser et al., 1983)
	Nemipteridae	*Nemipterus peronii*	H	(Young and Martin, 1985)
		Pentapodus porosus	H	(Young and Martin, 1985)
		Scolopsis bilineatus	G	(Young and Martin, 1985)
		Scolopsis monogramma	G	(Young and Martin, 1985)
		Scolopsis taeniopterus	G	(Young and Martin, 1985)
	Nototheniidae	*Eleginops maclovinus* (Cuv. and Val. 1830)	A	(Calvo et al., 1992)
	Percophidae	*Matsubaraea fusiforme*	A?	(Noichi et al., 1991)
	Pinguipedidae	*Parapercis snyderi*	G	(Kobayashi et al., 1993a; Ohnishi et al., 1997)
	Polynemidae	*Polydactylus indicus*	H	(Kagwade, 1976)
		Polydactylus sexfilis	A	(Szyper et al., 1991)
	Pomacanthidae	*Centropyge ferrugatus*	G	(Sakai, 1997; Sakai and Kohda, 1997)
		Centropyge interruptus	G	(Moyer and Nakazono, 1978a; Moyer and Zaiser, 1984)
		Centropyge multispinis	G	(Bruce, 1980)

Order	Family	Species	Type	References
		Centropyge potteri	G	(Lutnesky, 1988, 1989, 1994, 1996)
		Centropyge tibicen	G	(Moyer and Zaiser, 1984)
		Genicanthus bellus	G	(Hioki et al., 1995)
		Genicanthus caudovittatus	G	(Bruce, 1980)
		Genicanthus lamarck	G	(Suzuki et al., 1979)
		Genicanthus semifasciatus	G	(Shen and Liu, 1976; Suzuki et al., 1979)
		Genicanthus watanabei	G	(Hioki et al., 1995)
		Holacanthus tricolor	G	(Hourigan and Kelley, 1985)
	Pomacentridae	*Amphiprion akallopisos*	A	(Fricke, 1979)
		Amphiprion bicinctus	A	(Fricke, 1983)
		Amphiprion clarkii	A	(Moyer and Nakazono, 1978b; Ochi, 1989; Hattori, 1991, 1994; Hattori and Yanagisawa, 1991a,b)
		Amphiprion frenatus	A	(Moyer and Nakazono, 1978b; Truslé Sicard and Reinboth, 1990; Hattori, 1991; Truslé Sicard et al., 1992, 1994; Nakamura et al., 1994)
		Amphiprion melanopus	A	(Godwin and Thomas, 1993; Godwin, 1994a,b; Elofsson et al., 1997)
		Amphiprion ocellaris	A	(Moyer and Nakazono, 1978b)
		Amphiprion perideraion	A	(Moyer and Nakazono, 1978b)
		Amphiprion polymnus	A	(Moyer and Nakazono, 1978b)
		Amphiprion sandaracinos (*Amphiprion sandracinos*)	A	(Moyer and Nakazono, 1978b)
		Dascyllus aruanus	G	(Coates, 1982; Godwin, 1995)
		Dascyllus marginatus	G	(Godwin, 1995)
		Dascyllus reticulatus (Richardson)	G	(Schwarz and Smith, 1990)
	Pseudochromidae	*Anisochromis straussi*	G	(Springer et al., 1978)
	Scaridae	*Calotomus carolinus*	G	(Robertson et al., 1982)
		Calotomus japonicus	G	(Kusen and Nakazono, 1991)
		Calotomus spinidens	G	(Robertson et al., 1982)
		Chlorurus gibbus	G	(Choat et al., 1996)
		Chlorurus sordidus (*Scarus sordidus*)	G	(Yogo, 1985; Choat et al., 1996)
		Cryptotomus roseus	G	(Robertson and Warner, 1978)
		Scarus collana (*Scarus gharadaqensis*)	G?	(Bebars, 1978)
		Scarus frenatus	G	(Choat et al., 1996)
		Scarus iserti (*Scarus croicensis*)	G	(Robertson and Warner, 1978)
		Scarus niger	G	(Choat et al., 1996)
		Scarus psittacus	G	(Choat et al., 1996)
		Scarus rivulatus	G	(Choat et al., 1996)
		Scarus schlegeli (*Scarus schelgeli*)	G	(Choat et al., 1996)
		Scarus taeniopterus	G	(Robertson and Warner, 1978)
		Scarus vetula	G	(Robertson and Warner, 1978)

Order	Family	Species	Type	References
		Sparisoma atomarium	G	(Robertson and Warner, 1978)
		Sparisoma aurofrenatum	G	(Robertson and Warner, 1978; Clavijo, 1982)
		Sparisoma chrysopterum	G	(Robertson and Warner, 1978)
		Sparisoma radians	G	(Robertson and Warner, 1978)
		Sparisoma rubripinne	G	(Robertson and Warner, 1978)
		Sparisoma viride	G	(Robertson and Warner, 1978; Cardwell, 1990; Cardwell and Liley, 1991; Koltes, 1993)
	Serranidae	*Anthias taeniatus* (*Pseudanthias taeniatus*)	G?	(Katayama, 1978)
		Centropristis striata	G	(Shepherd and Idoine, 1993)
		Cephalopholis cruentata (*Epinephelus cruentatus*)	G	(Nagelkerken, 1979)
		Cephalopholis taeniops	G	(Siau, 1994)
		Diplectrum formosum	S	(Gomez Gaspar, 1985)
		Epinephelus aeneus	G	(Bruslé and Bruslé, 1976; Hassin et al., 1997)
		Epinephelus akaara	G	(Tanaka et al., 1990a,b; Lee, 1995)
		Epinephelus bruneus	G	(Lee, 1995)
		Epinephelus coioides (*Epinephelus suillus*)	G	(Tan-Fermin, 1992; Tan-Fermin et al., 1994; Sheaves, 1995; Quinitio et al., 1997)
		Epinephelus diacanthus	G	(Chen et al., 1980)
		Epinephelus guttatus	G	(Shapiro et al., 1993a,b, 1994; Sadovy et al., 1994)
		Epinephelus malabaricus	G	(Sheaves, 1995)
		Epinephelus marginatus (*Epinephelus gauza*)	G	(Bruslé and Bruslé, 1976; Chauvet, 1988; Mandich, 1998)
		Epinephelus morio	G	(Johnson and Thomas, 1995; Coleman et al., 1996; Johnson et al., 1998)
		Epinephelus niveatus	G	(Moore and Labisky, 1984)
		Epinephelus polyphekadion (*Epinephelus microdon*)	G	(Bruslé et al., 1989; Debas et al., 1989)
		Epinephelus septemfasciatus	G	(Lee, 1995)
		Epinephelus striatus	G *	(Jory and Iversen, 1989; Colin, 1992; Sadovy and Colin, 1995)
		Epinephelus tauvina	G	(Lee et al., 1995)
		Epinephelus rivulatus	G	(Mackie, 2000)
		Estriatus sp.	G	(Jory and Iversen, 1989)
		Hemanthias vivanus (Jordan and Swain)	G	(Hastings, 1981)
		Hypoplectrust nigricans	S	(Fischer and Petersen, 1987)
		Hypoplectrodes huntii (*Ellerkeldia huntii* (Hector))	G	(Jones, 1980b)

Order	Family	Species	Type	References
		Hypoplectrodes maccullochi	G	(Webb and Kingsford, 1992)
		Mycteroperca bonaci	G	(Jory and Iversen, 1989; Garcia Cagide and Garcia, 1996)
		Mycteroperca interstitialis	G	(Bullock and Murphy, 1994)
		Mycteroperca microlepis	G	(Roberts and Schlieder, 1983; Coleman et al., 1996)
		Mycteroperca phenax	G	(Coleman et al., 1996)
		Mycteroperca rubra	G	(Siau and Boauain, 1994)
		Mycteroperca venenosa	G	(Garcia Cagide and Garcia, 1996)
		Paralabrax humeralis (Valenciennes)	G	(Borquez et al., 1988)
		Paralabrax maculatofasciatus	G	(Hastings, 1989)
		Plectropomus leopardus (Lacepede)	G	(Ferreira and Russ, 1995)
		Plectropomus maculatus	G	(Ferreira, 1993)
		Pseudanthias bicolor (*Anthias bicolor*)	G	(Howe, 1996)
		Pseudanthias squamipinnis (*Anthias squamipinnis* or *Franzia squamipinnis*)	G	(Fishelson, 1970; Shapiro, 1979, 1980, 1981b,c, 1986, 1988; Yogo, 1985)
		Serranus baldwini	S	(Leonard, 1993)
		Serranus cabrilla	S	(Reinboth, 1979)
		Serranus fasciatus	G	(Petersen, 1990)
		Serranus scriba	S	(Abd-el-Aziz and Ramadan, 1990; Siau and Bouain, 1994)
		Serranus subligarius	S	(Oliver, 1991, 1997; Oliver et al., 1995)
		Serranus tigrinus	S	(Pressley, 1981)
		Serranus tortugarum	S	(Fischer and Petersen, 1987; Petersen and Fischer, 1996)
	Sparidae	*Acanthopagrus australis*	A	(Pollock, 1985; Buxton and Garratt, 1990)
		Acanthopagrus berda	A	(Garratt, 1993; Tobin et al., 1997)
		Acanthopagrus bifasciatus	A	(Buxton and Garratt, 1990)
		Acanthopagrus butcheri	G	(Rowland and Snape, 1994)
		Acanthopagrus latus	G	(Buxton and Garratt, 1990)
		Acanthopagrus schlegeli	A	(Chang and Yueh, 1990a; Chang et al., 1994, 1995b; Chang and Lin, 1998)
		Boops boops	G	(Gordo, 1995)
		Calamus leucosteus	G	(Waltz et al., 1982)
		Calamus nodosus	G	(Horvath et al., 1990)
		Cheimerius nufar	H	(Coetzee, 1983)
		Chrysoblephus cristiceps	G	(Buxton, 1990; Buxton and Garratt, 1990)
		Chrysoblephus laticeps	G	(Buxton, 1990; Buxton and Garratt, 1990)

Order	Family	Species	Type	References
		Chrysoblephus puniceus	G	(Garratt, 1986; Buxton and Garratt, 1990; Garratt, 1993)
		Crysophrys major	G	(Huang et al., 1974)
		Dentex gibbosus	GA	(Buxton and Garratt, 1990; Pajuelo and Lorenzo, 1995)
		Dentex tumifrons (*Taius tumifrons*)	G	(Buxton and Garratt, 1990)
		Diplodus annularis	A	(Buxton and Garratt, 1990)
		Diplodus sargus capensis	A	(Mann and Buxton, 1998)
		Diplodus sargus sargus (*Diplodus sargus*)	A	(Micale and Perdichizzi, 1994)
		Diplodus sargus kotschyi	A	(Abou-Seedo et al., 1990)
		Lithognathus aureti	A	(Buxton and Garratt, 1990)
		Lithognathus mormyrus	A	(Besseau and Faliex, 1989; Buxton and Garratt, 1990; Kraljevic et al., 1995)
		Pachymetopon aenum	G	(Buxton and Clarke, 1986; Buxton and Garratt, 1990)
		Pagellus acarne	A	(Reinboth, 1979; Lamrini, 1986; Reinboth et al., 1986)
		Pagellus bogaraveo	AS?	(Buxton and Garratt, 1990; Krug, 1990)
		Pagellus erythrinus	G	(Buxton and Garratt, 1990; Ghorbel, 1996)
		Pagrus auriga (*Sparus caeruleosticus*)	G	(Alekseev, 1982a, 1983)
		Pagrus caeruleostictus (*Pagrus ehrenbergi*, *Sparus ehrenbergi*)	G	(Alekseev, 1982a, 1983)
		Pagrus major (*Chrysophrys major*)	H	(Matsuyama et al., 1988)
		Pagrus pagrus (*Pagrus orphus*, *Sparus pagrus*)	G	(Alekseev, 1982a, 1983)
		Pterogymnus laniarus	G	(Buxton and Garratt, 1990)
		Rhabdosargus globiceps	G	(Buxton and Garratt, 1990)
		Rhabdosargus sarba	A	(Yeung and Chan, 1985a, 1987a,b,d; Chan and Yeung, 1989)
			G	(Yeung and Chan, 1985b)
		Sarpa salpa	A	(Buxton and Garratt, 1990)
		Sparidentex hasta (Valenciennes)	A	(Kime et al., 1991; Al-Marzouk et al., 1994)
		Sparus aurata	A	(Eckstein et al., 1978; Zohar et al., 1978; Bruslé Sicard and Fourcault, 1997)
		Sparus caeruleosticus	A	(Alekseev, 1982b)
		Sparus ehrengergi	A	(Alekseev, 1982b)
		Sparus latus (Houttuyn) (*Aacanthopagrus latus*)	A	(Hong et al., 1991)
		Sparus macrocephalus	A	(Ruan et al., 1996)

Order	Family	Species	Type	References
		Sparus pagrus	A	(Alekseev, 1982b)
		Spondyliosoma cantharus (*Cantharus cantharus*)	G	(Buxton and Garratt, 1990; Dulcic and Kraljevic, 1996)
		Taius tunifrons	A	(Aoyama, 1955)
	Terapontidae	*Terapon jarbua* (*Therapon jarbua*)	G	(Liu, 1979)
	Trichonotidae	*Trichonotus filamentosus*	G	(Kusen et al., 1991)
Siluriformes	Heteropneustidae	*Heteropneustes fossilis*	H	(Saxena and Bhatia, 1977)
Stomiiformes	Gonostomatidae	*Cyclothone atraria*	A	(Miya and Nemoto, 1985; Badcock, 1986)
		Cyclothone microdon	A	(Miya and Nemoto, 1985; Badcock, 1986)
		Gonostoma bathyphilum	A	(Badcock, 1986)
		Gonostoma elongatum	A	(Miya and Nemoto, 1985; Badcock, 1986)
		Gonostoma gracile	A	(Miya and Nemoto, 1985; Badcock, 1986)
Synbranchiformes	Synbranchidae	*Monopterus albus*	G	(Chan and Yeung, 1989; Tao et al., 1993; Yeung et al., 1993a,b,c; Xiao and Liu, 1995)
		Synbranchus marmoratus	G	(Lo Nostro and A, 1996; Ravaglia et al., 1997)

G, protogynous; A, protandrous; S, simultaneous/synchronous; H, bisexual juvenile gonad or hermaphroditism requiring further investigation; * mostly gonochoristic. This table provides a broad overview, but is not inclusive of all known hermaphroditic species.

Appendix B. Karyotypes where the presence of sex chromosomes has been investigated in fish

Order	Family	Species	2*n*	Sex chromosome system	References
Rajiformes	Dasyatidae	*Dasyatis sabina*	68	XY	(Donahue, 1974; Kirpichnikov, 1981)
		Dasyatis say	68	XY	(Maddock and Schwartz, 1996)
	Rajidae	*Raja eglanteria*		ND	(Maddock and Schwartz, 1996)
	Rhinobatidae	*Platyrhinoidis triseriata*		XY	(Maddock and Schwartz, 1996)
		Rhinobatus productus		XY	(Maddock and Schwartz, 1996)
Acipenseformes	Acipenseridae	*Acipenser transmontanus*	265–276	ND *	(Van Eenennaam et al., 1998b)
		Acipenser medirostris	234–263	ND	(Van Eenennaam et al., 1999a)
Anguilliformes	Anguillidae	*Anguilla anguilla*	38	ZW	(Passakas, 1981)
		Anguilla anguilla	38	ND	(Cau et al., 1992; Gomez et al., 1993)

Order	Family	Species	2*n*	Sex chromosome system	References
		Anguilla japonica	38	ZW	(Park and Kang, 1979)
		Anguilla rostrata	38	ZW	(Passakas, 1981)
	Congridae	*Astroconger myriaster*	38	ZW	(Park and Kang, 1979)
	Muraenidae	*Muraena helaena*	42	ND	(Salvadori et al., 1989)
		Gymnothorax eurostus	42	XY	(Ojima, 1985)
	Ophichthidae	*Muraenichthys gymnotus*		Y–A	(Murofushi and Yosida, 1984)
Atheriniformes	Atherinidae	*Basilichthys australis*		ND	(Gajardo and Arratia, 1981)
		Basilichthys microlepidotus		ND	(Gajardo and Arratia, 1981)
		Odontesthes de Bueni		ND	(Gajardo and Arratia, 1981)
		Odontesthes mauleanum		ND	(Gajardo and Arratia, 1981)
Aulopiformes	Chlorophthalmidae	*Chlorophthalmus albatrossis*	36	ND	(Ota et al., 2000)
	Synodontidae	*Saurida undosquamis*	48	ZW	(Nishikawa and Sakamoto, 1978)
		Saurida. elongata	48	ZW	(Nishikawa and Sakamoto, 1978; Ota et al., 2000)
		Synodus ulae	48	ZW	(Ota et al., 2000)
		Synodud hoshinonus	48	ZW	(Ota et al., 2000)
		Trachinocephalus myops	26/27	$ZZ-ZW_1W_2$	(Ota et al., 2000; Ueno et al., 2001)
Beloniformes	Adrianichthyidae	*Oryzias latipes*	48	ND; XY	(Uwa and Ojima, 1981; Matsuda et al., 1998)
Beryciformes	Berycidae	*Beryx splendens*		Y–A	(Ojima and Kikuno, 1986)
	Melamphaeidae	*Melamphaes parvus*	50	XY	(Chen, 1969)
		Scopeloberyx mizolepis bispinosus	46	XY	(Chen, 1969)
		Scopeloberyx robustus	42	XY	(Chen, 1969)
		Scopelogadus mizolepis		XY	(Ebeling and Chen, 1970)
Carchariniformes	Carcharhinidae	*Galeocerdo cuiver*	86	ND	(Maddock and Schwartz, 1996)
Characiformes	Anostomidae	*Leporinus lacustris*	54	XY	(Galetti et al., 1981)

Order	Family	Species	2*n*	Sex chromosome system	References
		Leporinus silvestrii, *L. obtusidens*	54	ZW	(Galetti et al., 1981)
		Leporinus lacustris	54	ND	(Mestriner et al., 1995)
		Leporinus elongatus	54	ZW	(Galetti and Foresti, 1986)
	Characidae	*Acestrorhynchus lacustris*	50	ND	(Das Neves Falcao and Bertollo, 1985)
		Aphyocharax difficilis	50	ND	(Souza et al., 1995)
		Astyanax bimaculatus	50	ND	(Morelli et al., 1983)
		Astyanax fasciatus	46, 48	ND	(Morelli et al., 1983)
		Astyanax schubarti	36	ND	(Morelli et al., 1983)
		Astyanax scabripinnis paranae	50	ND	(Morelli et al., 1983)
		Astyanax scabripinnis	50	ND	(Stange and Almeida-Toledo, 1993)
		Galeocharax knerii	52	ND	(Das Neves Falcao and Bertollo, 1985)
		Oligosarcus hepsetus, *O. macrolepis*, *O. pintoi*	50	ND	(Das Neves Falcao and Bertollo, 1985)
		Triportheus guentheri, *T. flavus*, *T. elongatus*, *T. albus*, *T. paranense*, *T. signatus*	52	ZW	(Bertollo and Cavallaro, 1992; Sanchez and Jorge, 1999; Artoni et al., 2001)
		Triportheus pictus, *T. culter*	52	ND	(Sanchez and Jorge, 1999; Artoni et al., 2001)
	Characinidae	*Characidium fasciatum*	50–54	ZW	(Maistro et al., 1998)
	Curimatidae	*Semaprochilodus taeniurus*	54	ZW	(Feldberg et al., 1987; Val and de Almeida-Val, 1995)
	Erythrinidae	*Hoplias lacerdae*	50	XY	(Bertollo et al., 1978)

Order	Family	Species	2*n*	Sex chromosome system	References
		Hoplias malabaricus	40	ND	(Dergam and Bertollo, 1990)
		Hoplias malabaricus	40, 42	ND	(Lopez and Fenocchio, 1994)
		Hoplias malabaricus	40/39	Y–A, XY	(Bertollo and Mestriner, 1998; Bertollo et al., 2000; Born and Bertollo, 2000)
	Hemiodontidae	*Apareiodon affinis*	54/55	ZZ/ZW_1W_2	(Moreira et al., 1985; de Jesus et al., 1999)
		Apareiodon ibitiensis, *A. piracicabae*	54	ND	(Moreira et al., 1985)
		Parodon tortuosus	54	ND	(Moreira et al., 1985; de Jesus and Moreira-Filho, 2000)
		Parodon hilarii	54	ZW	(Moreira-Filho et al., 1993; de Jesus and Moreira-Filho, 2000)
	Serrasalmindae	*Serrasalmus spilopleura*	60	ND	(Nakayama et al., 2000)
Clupeiformes	Clupeidae	*Brevoortia aurea*	46	Y–A	(Brum, 1996)
	Notopteridae	*Notopterus notopterus*	42	ND	(Sishi and Singh, 1983)
Cypriniformes	Balitoridae	*Noemacheilus botia*	50	ND	(Rishi et al., 1977)
	Balitoridae	*Nemachilus barbatulus*	50	ND	(Sofradzija and Vukovic, 1979)
	Cobitidae	*Botia dario*, *B. hymenophysa*	90	ND	(Rishi and Haobam, 1984)
		Cobitis taenia	48	ND	(Boron, 1995)
		Cobitis taenia	50/49	Y–A	(Saitoh, 1989)
		Lepidocephalichthys berdmorei	62	ZW	(Rishi and Haobam, 1984)
		Lepidocephalichthys guntea	51/52	ZO/ZZ	(Sharma and Tripathi, 1988)
		Sabanejewia larvata	50	ND	(Lodi and Marchionni, 1980)
	Cyprinidae	*Amblypharyngodon calbasumola*	50	ND	(Manna and Khuda-Bukhsh, 1977)
		Barilius bendelisis	50	ND	(Khuda-Bukhsh, 1979b)

Order	Family	Species	2*n*	Sex chromosome system	References
		Barilius shacra	52	XY	(Khuda Bukhsh et al., 1992)
		Barilius barna, *B. tileo*	50	ND	(Khuda Bukhsh et al., 1992)
		Brachydanio (*Danio*) *rerio*	50	ND *	(Rishi et al., 1977; Sharma et al., 1998)
		Carassius carassius auratus	100	XY	(Kirpichnikov, 1981)
		Carassius auratus	166	ND *	(Chen et al., 1996)
		Carassius auratus gibelio	156	ND *	(Shen et al., 1983)
		Catla catla	50	ND	(Manna and Khuda-Bukhsh, 1977)
		Chela bacaila	50	ND	(Manna and Khuda-Bukhsh, 1977)
		Cirrhina reba	48	ND	(Manna and Khuda-Bukhsh, 1977)
		Cirrhina mrigala, *C. reba*	50	ND	(Rishi, 1981)
		Clenopharyngodon idellus	48	ND	(Manna and Khuda-Bukhsh, 1977)
		Danio (*Brachidanio*) *rerio*	50	ZW	(Rishi et al., 1977; Sharma et al., 1998)
		Esomus danrica	50	ND	(Manna and Khuda-Bukhsh, 1977)
		Hypaphthalmichthys molitrix	48	ND	(Manna and Khuda-Bukhsh, 1977)
		Labeo bata	50	ND	(Manna and Khuda-Bukhsh, 1977)
		Labeo rohita, *L. calbasu*	50	ND	(Krishnaja and Rege, 1979)
		Labeo cerulaeus	48	ND	(Rishi, 1981)
		Leuciscus carolitertii	50	ZW	(Collares-Pereira et al., 1998)
		Leuciscus pyrenaicus	50	ZW	(Collares-Pereira et al., 1998)
		Megalobrama amplycephala	48	ND	(Lin, 1985)
		Notropis ardens, *N. braytoni*, *N. proserpinus*	50	ND	(Amemiya and Gold, 1987)

Order	Family	Species	2*n*	Sex chromosome system	References
		Parabramis pekinensis	48	ND	(Lin, 1985)
		Puntius sophore	48	ND	(Rishi et al., 1977)
		Puntius sophore	48	ND	(Sharma et al., 1990)
		Puntius ticto	50	ND	(Sharma et al., 1990)
		Puntius sarana	50	ND	(Rishi, 1981)
		Rasbora buchanani	50	ND	(Manna and Khuda-Bukhsh, 1977)
		Rohlee colio	48	ND	(Manna and Khuda-Bukhsh, 1977)
		Scardinius erythrophthalmus	50	ZW/ZW′/ZZ	(Koehler et al., 1995)
		Schizothorax richardsonii, *S. kumaonensis*	98	ND	(Lakra et al., 1997)
		Tor putitora, *T. khudree*	100	ND	(Lakra, 1996)
		Vimba vimba	48	XY	(Rudek, 1974; Kirpichnikov, 1981)
Cyprinodontiformes	Aplocheilidae	*Aplocheilus panchax*	38	ZW	(Khuda-Bukhsh, 1979a)
		Nothobranchius guentheri	36/35	Y–A	(Ewulonu et al., 1985)
		Pterolebias hoignei	46	XY	(Elder et al., 1991)
		Pterolebias zonatus	42	ND	(Elder et al., 1991)
	Cyprinodontidae	*Germenella pulchra*	50/49	Y–A	(Levin and Foster, 1972)
		Megupsilon aporus	48/47	Y–A	(Uyeno, 1973)
		Valencia lozanoi	48	ND	(Delgado Bermejo and Moreno Millan, 1988)
		Unnamed mexican cyprinodontid	48/47	Y–A	(Uyeno and Miller, 1971)
	Fundulidae	*Fundulus diaphanus*	48	XY	(Chen and Ruddle, 1970; Ebeling and Chen, 1970)
		Fundulus parvipinnis	48	XY	(Chen and Ruddle, 1970; Ebeling and Chen, 1970)
	Goodidae	*Allodontichthys hubbsi*	42/41	Y–A	(Miller and Uyeno, 1980)
	Poeciliidae	*Boleophthalmus boddaerti*	46	ZW	(Subrahmanyam, 1969)
		Gambusia affinis affinis, *G. hurtadoi*, *G. nobilis*	48	ZW	(Chen and Ebeling, 1968)

Order	Family	Species	2n	Sex chromosome system	References
		Gambusia affinis holbrooki	48	ZW	(Ojima, 1985)
		Gambusia affinis holbrooki	48	ND	(Black and Howell, 1979; Sharma and Tripathi, 1982; Krishnaja and Rege, 1983)
		Gambusia gaigei	48	ZW	(Campos and Hubbs, 1971)
		Gambusia puncticulata puncticulata	48	ZW	(Rab, 1984)
		Glaridichthys falcatus, *Quintana atrizona*	48	ND	(Rab, 1984)
		Limia vittata	46	ND	(Rab, 1984)
		Poecilia formosa	46	ND	(Sola et al., 1992b)
		Poecilia mexicana mexicana	46	ND	(Sola et al., 1992a)
		Poecilia reticulata	46	ND *	(Lodi, 1978)
		Poecilia reticulata	46	XY	(Nanda et al., 1990)
		Poecilia latipinna	48	ZW	(Sola et al., 1990)
		Poecilia latipunctata	46	ND	(Galetti and Rasch, 1993)
		Poecilia sphenops	46	ZW	(Rishi, 1976a; Haaf and Scmid, 1984 10731; Ojima, 1985; Manna, 1989; Nanda et al., 1993)
		Poecilia velifera	46	ZW	(Nanda et al., 1993)
		Xiphophorus maculatus	48	XY	(Foerster and Anders, 1977)
		Xiphophorus variatus, *X. montezumae cortezi*, *X. helleri strigatus*, *X. helleri guentheri*, *X. helleri helleri*	48	ND	(Foerster and Anders, 1977)
Gadiformes	Gadidae	*Theragra chalcogramma*	44	ND	(Ishii and Yabu, 1985)
		Eleginus navaga	26	ND	(Klinkhardt, 1994)
		Gadus morhua marisalbi	46	ND	(Klinkhardt, 1994)
	Lotidae	*Lota lota*	48	ND	(Klinkhardt, 1994)
Gasterosteiformes	Gasterosteidae	*Apeltes quadracus*	46	ZW	(Chen and Reisman, 1970; Ebeling and Chen, 1970)

Order	Family	Species	2*n*	Sex chromosome system	References
		Gasterosteus aculeatus	42	ND	(Klinkhardt and Buuk, 1990a)
		Gasterosteus wheatlandi	42	XY	(Chen and Reisman, 1970; Ebeling and Chen, 1970)
		Pungitius pungitius	42	ND	(Klinkhardt and Buuk, 1990a)
Gobiesociformes	Gobiesocidae	*Diademichthus lineatus*	48/47	XX/XO	(Arai et al., 1976)
		Lepadogaster candollei	46	XY	(Thode, 1987)
Gymnotiformes	Sternopygidae	*Eigenmannia* spp.	32/31	X_1X_2/Y	(de Almeida Toledo et al., 1984; Almeida-Toledo et al., 2000a)
	Hypopomidae	*Brachyhypopomus pinnicaudatus*	42/41	X_1X_2/Y	(Almeida-Toledo et al., 2000b)
Myctophiformes	Myctophidae	*Lampanyctus ritteri*	48/47	XX/XO	(Chen, 1969)
		Parvilux ingens	50/49	XX/XO	(Chen, 1969)
		Symbolophorus californiensis	48	XY	(Chen, 1969)
	Neoscopelidae	*Scopelengys tristis*	48	XY	(Chen, 1969)
Osteoglossiformes	Osteoglossidae	*Osteoglossum bicirrhosum*	56	XY	(Uyeno, 1973)
	Mormyridae	*Marcusenius brachistius*	48	XY	(Uyeno, 1973)
Perciformes	Belontiidae	*Betta splendens*	42	ND *	(Ratanatham and Patinawin, 1979)
		Colisa fasciatus	48	ZW	(Rishi, 1975; Sharma and Tripathi, 1988)
		Colisa fasciatus	48	ND	(Manna and Prasad, 1977)
		Trichogaster labiosus	48	ND	(Manna and Prasad, 1977)
		Colisa lalius	46/45	ZZ/ZO	(Rishi, 1976a)
	Blenniidae	*Parablennius* (*Blennius*) *tentacularis* Brunnich	47/48	Y–A	(Carbone et al., 1987; Caputo et al., 2001)
		Aidablennius sphynx	48	ND	(Caputo et al., 2001)
		Blennius ocellaris	48	ND	(Caputo et al., 2001)
		Lypophris adriaticus	48	ND	(Caputo et al., 2001)
		Lypophris pavo	48	ND	(Caputo et al., 2001)
		Lypophris trigloides	48	ND	(Caputo et al., 2001)
		Parablennius gattorugine	48	ND	(Caputo et al., 2001)
		Parablennius ponticus	48	ND	(Caputo et al., 2001)

Order	Family	Species	2*n*	Sex chromosome system	References
		Parablennius sanguinolentus	48	ND	(Caputo et al., 2001)
	Callionymidae	*Callionymus punctatus*, *C. doryssus*	38	XY	(Murofushi et al., 1984)
		Callionymus beniteguri, *C. ornatipinnis*	38/37	Y–A	(Murofushi et al., 1984)
		Callionymus sagitta	38	XY	(Dass, 1983)
	Carangidae	*Salar kalla*	48	ZW	(Dass, 1983)
		Seriola dumerili	48, 47	ND	(Vitturi et al., 1986)
		Trachurus trachurus, *T. mediterraneus*	48	ND	(Caputo et al., 1996a)
		Trichnotus ovatus		ZW	(Dass, 1983)
	Centrarchidae	*Lepomis cyanellus*	48	XX/XO	(Becak et al., 1966)
		Micropterus punctulatus, *M. treculi*	46	ND	(Thompson et al., 1978)
	Centropomidae	*Coreoperca herzi*	48	ND	
		Coreoperca kawamebari	48	ND	
		Siniperca schezeri	48	ND	
	Channichthyidae	*Chaenodraco wilsoni*, *Pagetopsis macropterus*, *Chionodraco myersi*, *C. hamatus*	48/47	Y–A	(Morescalchi et al., 1992b)
		Channichthys rhinoceratus, *Cryodraco antarcticus*, *Neopagetopsis ionah*	48	ND	(Morescalchi et al., 1992b)
	Channidae	*Channa stewartii*	104	ND	(Rishi and Haobam, 1984)
	Cichlidae	*Geophagus brasiliensis*	48	XY	(Michele and Takahashi, 1977)
		Sarotherodon galilaeus, *Tilapia zillii*, *Oreochromis aureus*, *O. mossambicus*, *O. spilurus*, *O. nyasalapia*, *O. macrochir*	44	ND *	(Majumdar and McAndrew, 1986)
		Oreochromis niloticus	44	XY	(Carrasco et al., 1999)
		Oreochromis rendalli	44	ND	(Foresti et al., 1983; Swanepoel et al., 1992)

Order	Family	Species	2*n*	Sex chromosome system	References
		Oreochromis sparrmanii	42	ND	(Swanepoel et al., 1992)
	Eleotridae	*Eleotris pisonis*	44	ZW	(Uribe-Alcocer et al., 1994)
	Gobiidae	*Apocryptichthus cantoris*	46	XY	(Dass, 1983)
		Boleophthalmus boddaerti	46	ZW	(Kirpichnikov, 1981)
		Dormitator maculatus	46	ND	(del Carmen Maldonado et al., 1985)
		Gobiodon citrinus	44/43	XX/XO	(Arai and Sawada, 1974)
		Gobiomorus dormitor	48	ND	(del Carmen Maldonado et al., 1985)
		Gobionellus shufeldti	48/47	Y–A	(Pezold, 1984)
		Gobius bucchichi	44	ND	(Thode et al., 1983)
		Gobius cruentatus	46	ND	(Thode et al., 1983)
		Gobius cobitis, *G. paganellus*	46	XY	(Thode et al., 1983)
		Gobius bucchichi	40	XY	(Thode et al., 1983)
		Luciogobius guttatus	44	ND	(Mao et al., 1993)
		Mogrunda obscura	46	XY	(Nogusa, 1960)
		Neogobius kessleri	30/29	Y–A	(Vasil'ev and Vasil'eva, 1992)
		Proterorhinus marmoratus	46	XY	(Rab, 1985)
		Tridentiger trigonocephalus	44	ND	(Mao et al., 1993)
		Synechogobius ommaturus	44	XY	(Wang and Zhao, 1994)
		Zosterisessor ophiocephalus	46	ND	(Caputo et al., 1996b)
	Haemulidaee	*Haemulon aurolineatum*	48	ND	(Duran Gonzalez et al., 1990)
	Istiophoridae	*Tetrapturus albidus*	48	XY	(Duran Gonzalez and Laguarda Figueras, 1992)
	Labridae	*Coris julis*	46/45	XX/XO	(Cataudella et al., 1973)
		Coris julis	46	XY **	(Duchac et al., 1982)
		Coris julis	48	ND	(Vitturi et al., 1988)
	Microdesmidae	*Parioglossus raoi*	46	ND	(Webb, 1986)
	Monodactylidae	*Monodactylus sebae*	48/47	Y–A	(Suzuki et al., 1988a)
		Monodactylus argenteus	48	ND	(Suzuki et al., 1988a)
	Moronidae	*Dicentrarchus labrax*	48	XY *	(Cano et al., 1996)

Order	Family	Species	2*n*	Sex chromosome system	References
	Mugilidae	*Mugil cephalus*	48	ND	(Rossi et al., 1996)
	Nandidae	*Badis badis*	52	ND	(Sharma and Tripathi, 1984)
		Nandus nandus	48	ND	(Manna and Prasad, 1977)
	Nototheniidae	*Pagothenia hansoni*, *P. borchgrevinki*	46/45	Y–A	(Morescalchi et al., 1992a)
		Trematomus newnesi	46/45	Y–A	(Morescalchi et al., 1992a)
		Trematomus nicolai	58/57	Y–A	(Morescalchi et al., 1992a)
	Percidae	*Accrina cernua*	48	XY	(Lieder, 1963)
		Parapercis sexfasciata	48	XY	(Ojima et al., 1984)
		Perca fluviatilis	48	ND	(Klinkhardt and Buuk, 1991)
	Pomacentridae	*Dascyllus trimaculatus*	48/47	XX/XO	(Arai and Inoue, 1976)
	Pholidae	*Enedrias nebulosus*	26	ND	(Mao and Jin, 1994)
	Scatophagidae	*Scatophagus argus*	48	XY	(Khuda-Bukhsh and Manna, 1977; Khuda-Bukhsh and Chakrabarti, 1999)
	Serranidae	*Epinephelus guttatus*	48	ND	(Ruiz Carus, 1983)
		Epinephelus tauvina	48	ZW	(Dass, 1983)
	Sparidae	*Sparus macrocephalus*	48	ND	(Liu and Tian, 1991)
		Pagrosomus major	48	ND	(Liu and Tian, 1991)
	Stichaeidae	*Alectrias benjamini*	48	ND	(Mao and Qiu, 1996)
Pleuronectiformes	Bothidae	*Bothus podas*	38	XY	(Vitturi et al., 1993b)
	Cynoglossidae	*Cynoglossus puncticeps*	40/39	ZZ/ZO	(Pense, 1965)
		Symphurus plagiusa	46/45	XX/XO	(LeGrande, 1975)
	Pleuronectidae	*Limanda limanda*	46	ND	(Klinkhardt, 1993)
		Platichthys flesus	48	ND	(Klinkhardt, 1993)
	Soleidae	*Microchirus ocellatus*	42	XY	(Vitturi et al., 1993a)
Salmoniformes	Argentinidae	*Argentina silus*	44	XY	(Ebeling et al., 1971)
	Bathylagidae	*Bathylagus milleri*	60	XY	(Chen, 1969)
		Bathylagus ochotensis	54	XY	(Chen, 1969)
		Bathylagus wesethi	36	XY	(Chen, 1969)
		Leuroglossus stilbius	60	XY	(Chen, 1969)
	Galaxiidae	*Galaxias platei*	30	XO	(Campos, 1972)

Order	Family	Species	2*n*	Sex chromosome system	References
	Salmonidae	*Coregonus sardinella*	80/81	XX/XY_1Y_2	(Frolov, 1990)
		Hucho hucho	82–84	XY	(Rab et al., 1994)
		Oncorhynchus kisutch	60	ND*	(Shelenkova, 1987)
		Oncorhynchus nerka	58/57	Y–A	(Thorgaard, 1978; Ueda and Ojima, 1984b)
		Oncorhynchus mykiss	58–63	XY**	(Thorgaard, 1977, 1983a; Ueda and Ojima, 1984a; Frolov, 1989; Colihueque et al., 2001)
		Salmo salar	58	ND*	(Bolla, 1987)
		Salvelinus fontinalis	84	ND	(Lee and Wright, 1981)
		Salvelinus namaycush	88	XY	(Phillips and Ihssen, 1985)
Scorpaeniformes	Cottidae	*Cottus gobio*	48	ND	(Vitturi and Rasotto, 1990)
		Cottus pollax	48	XY	(Nogusa, 1960; Kirpichnikov, 1981)
	Platycephalidae	*Platycephalus tuberculatus*	48	ND	(Nayak and Khuda Bukhsh, 1988)
	Sebastidae	*Sebastes taczanowskii*	48	ND	(Sasaki and Sakamoto, 1977)
	Tetrarogidae	*Hypodytes rubripinnis*	48/47	Y–A	(Ueno and Kang, 1992)
Siluriformes	Ageneiosidae	*Ageneiosus brevifilis*, *A. atronases*	56	ND	(Fenocchio and Bertollo, 1992)
	Ariidae	*Netuma barba*	56	XY	(Brum, 1996)
	Bagridae	*Mystus cavassius*	58	ND	(Khuda-Bukhsh et al., 1980)
		Mystus gulio	58	XY	(Dass, 1983)
		Mystus tengara	54	XY	(Das and Srivastava, 1973)
		Mystus tengara	54	ZW	(Rishi, 1973)
		Rita chrysea	54	ND	(Das and Kar, 1977)
	Callychthydae	*Callichthys callichthys* Linee	52–58	ND**	(Sanchez, 1996)
	Clariidae	*Clarias fuscus*	56	XY	(Luo et al., 1986)
		Clarias gariepinus	56	ZW*	(Ozouf Costaz et al., 1990)
		Clarias batrachus	50	ZW	(Pandey, 1997)
		Oncorhynchus tshawytscha	68	XY	(Stein et al., 2001)

Order	Family	Species	2*n*	Sex chromosome system	References
	Ictaluridae	*Ictalurus furcatus*	60	ND	(Marian and Krasznai, 1982)
		Notorus taylori	40	XY	(LeGrande, 1981)
	Loricariidae	*Loricariichthys platymetopon*	54	ZW	(Scavone and Julio, 1995)
		Hypostomus sp.	64	ZW	(Artoni et al., 1998)
		Microlepidogaster leucofrenatus	54–56	ZW	(Andreata et al., 1993)
		Pseudotocinclus tietensis	54	XY	(Andreata et al., 1992)
		Plecostomus ancistroides	68	XY	(Michele et al., 1977)
		Plecostomus macrops	68	XY	(Ojima, 1985)
	Mochokidae	*Hemisynodontis membranaceous*, *Synodontis bastiani*, *S. courteti*, *S. sorex*, *S. schall*, *S. budgetti*, *S. ocellifer*, *S. violaceus*	54	ZW	(Agnese et al., 1990)
		Synodontis filamentosus	56	ZW	(Agnese et al., 1990)
	Pimelodidae	*Imparfinis mirini*	58	ZW	(Vissotto et al., 1997)
		Rhamdia sapo	58,59	ND	(Valcarcel et al., 1993)
	Plotosidae	*Plotosus canius*	36	ND	(Rishi and Singh, 1983a)
	Schilbeidae	*Pseudeutropius atherinoides*	58	ND	(Rishi and Singh, 1983a)
	Siluridae	*Callichrous bimaculatus*	42/41	Y–A	(Rishi, 1976b)
		Silurus asotus	58	ND	(Kim et al., 1988)
		Wallago attu	86	ND	(Rishi and Singh, 1983b)
Stomiiformes	Sternoptychidae	*Sternoptyx diaphana*	36/35	XX/XO	(Chen, 1969)
Synbranchiformes	Mastacembelidae	*Mastacembelus pancalus*	48	ND	(Manna and Prasad, 1977)
		Macrognathus aculeatum	48	ND	(Manna and Prasad, 1977)
	Synbranchidae	*Monopterus albus*	24	XY	(Liu, 1983)
Syngnathiformes	Syngnathidae	*Hippocampus hippocampus*	44	ND	(Vitturi and Catalano, 1988)
		Hippocampus ramulosus	48	ND	(Vitturi and Catalano, 1988)
Tetraodontiformes	Balistidae	*Balistes carolinensis*	44	ND	(Thode et al., 1994)
		Rhinecanthus verrucosus, *R. echarpe*	44	XY	(Ojima, 1985)

Order	Family	Species	2*n*	Sex chromosome system	References
		Rhinecanthus aculeatus	44	XY	(Ojima, 1985)
		Odonus niger	42	XY	(Ojima, 1985)
	Monacanthidae	*Parika scaber*	40	ND	(Murofushi et al., 1989)
		Stephanolepis hispidus	34	Y–A	(Brum, 1996)
		Stephanolepis cirrhifer	32	Y–A	(Murofushi, 1980)
	Tetraodontidae	*Arothron immaculatus*	42	ND	(Choudhury et al., 1982)
		Arothron nigropunctatus	38/37	Y–A	(Ojima, 1985)
		Takifugu obscurus	44	ND	(Park et al., 1997)
	Triacanthidae	*Triacanthus brevirostris*	48/47	XX/XO	(Choudhury et al., 1982)
Zeiformes	Zeidae	*Zeus faber*	44/42	Y–A	(Vitturi et al., 1991a)

ND, sex chromosomes not detected; Y–A, Y–autosome fusion; *, genetic sex determination observed or different using other techniques (see Tables 1, 2, and 3); **, polymorphic. The species listed in this table are not intended to be inclusive of all karyotypically examined to date.

References

Abd-el-Aziz, S.H., Ramadan, A.A., 1990. Sexuality and hermaphroditism in fishes: I. Synchronous functional hermaphroditism in the serranid fish *Serranus scriba* L. Folia Morphol. 38, 86–100.

Abou-Seedo, F., Wright, J.M., Clayton, D.A., 1990. Aspects of the biology of *Diplodus sargus kotschyi* (Sparidae) from Kuwait Bay. Cybium 14, 217–223.

Abucay, J.S., Mair, G.C., Skibinski, D.O.F., Beardmore, J.A., 1999. Environmental sex determination: the effect of temperature and salinity on sex ratio in *Oreochromis niloticus* L. Aquaculture 173, 219–234.

Afonso, L.O.B., Barcellos, L.J.G., Leboute, E.M., Souza, S.M.G., 1994. Sex reversal of Nile tilapia *Oreochromis niloticus*, under laboratory conditions, using the hormone 17-alfa-methyltestosterone. Proceedings of the 4th Rio Grande Meeting of Aquaculture Experts Anais Do vol. 4, pp. 104–108.

Afonso, L.O.B., Campbell, P.M., Iwama, G.K., Devlin, R.H., Donaldson, E.M., 1997. The effects of the aromatase inhibitor fadrozole and two polynuclear aromatic hydrocarbons on sex-steroid secretion by ovarian follicles of coho salmon. Gen. Comp. Endocrinol. 106, 169–174.

Afonso, L.O., Iwama, G.K., Smith, J., Donaldson, E.M., 1999a. Effects of the aromatase inhibitor fadrozole on plasma sex steroid secretion and ovulation rate in female coho salmon, *Oncorhynchus kisutch*, close to final maturation. Gen. Comp. Endocrinol. 113, 221–229.

Afonso, L.O.B., Iwama, G.K., Smith, J., Donaldson, E.M., 1999b. Effects of the aromatase inhibitor fadrozole on plasma sex steroid secretion and oocyte maturation in female coho salmon (*Oncorhynchus kisutch*) during vitellogenesis. Fish Physiol. Biochem. 20, 231–241.

Afonso, L.O.B., Wassermann, G.J., de, O.R.T., 2001. Sex reversal in Nile tilapia (*Oreochromis niloticus*) using a nonsteroidal aromatase inhibitor. J. Exp. Zool. 290, 177–181.

Afonso, L.O.B., Smith, J.L., Ikonomou, M.G., Devlin, R.H., 2002. Y-chromosomal DNA markers for discrimination of chemical substance effects and effluent effects on sexual differentiation in salmon, Env. Health Perp. (in press).

Agnese, J.F., Oberdorff, T., Ozouf Costaz, C., 1990. Karyotypic study of some species of family mochokidae pisces siluriformes evidence of female heterogamety. J. Fish Biol. 37, 375–382.

Ahuja, M.R., Lepper, K., Anders, F., 1979. Sex chromosome aberrations involving loss and translocation of tumor-inducing loci in *Xiphophorus*. Experientia 35, 28–30.

Aida, T., 1921. On the inheritance of colour in a fresh-water fish *Aplocheilus latipes* Temminck and Aclegel, with special reference to sex-linked inheritance. Genetics 6, 554–573.

Aida, T., 1936. Sex reversal in *Aplocheilus latipes* and a new explanation of sex differentiation. Genetics 21, 136–153.

Aida, S., Arai, K., 1998. Sex ratio in the progeny of gynogenetic diploid marbled sole *Limanda yokohame* males. Fish. Sci. 64, 989–990.

Akhundov, M.M., Fedorov, K.E., 1990. Early gameto- and gonadogenesis in sturgeons: 1. Criteria of comparative assessment of gonad development in young individuals with reference to *Acipenser gueldenstaedti*. J. Ichthyol. 30, 963–973.

Akhundov, M.M., Fedorov, K.E., 1994. Effect of exogenous estradiol on the formation of ovaries in juvenile sterlet *Acipenser ruthenus*. Vopr. Ikhtiol. 34, 557–563.

Al-ablani, S.A., Phelps, R.P., 1997. Sex reversal in black crappie *Pomoxis nigromaculatus*: effect of oral administration of 17 alpha-methyltestosterone on two age classes. Aquaculture 158, 1–2.

Alekseev, F.E., 1982a. Hermaphroditism in sparid fishes (Perciformes, Sparidae): 1. Protogyny in porgies, *Pagrus pagrus*, *P. orphus*, *P. ehrenbergi* and *P. auriga*, from West Africa. J. Ichthyol. 22, 85–94.

Alekseev, F.E., 1982b. Hermaphroditism in sparid fishes (Perciformes, Sparidae): 1. Protogyny in porgies, *Pagrus pagrus*, *P. orphus*, *P. ehrenbergi*, and *P. auriga*, from West Africa. J. Ichthyol. 22, 85–94.

Alekseev, F.E., 1983. Hermaphroditism in porgies (Perciformes, Sparidae): 2. Sexual structure of the populations, mechanism of its formation and evolution in scups, *Pagrus pagrus*, *P. orphus*, *P. ehrenbergi*, and *P. auriga*. J. Ichthyol. 23, 61–73.

Allendorf, F.W., Thorgaard, G.H., 1984. Tetraploidy and the Evolution of Salmonid Fishes, Plenum Press, New York, pp. 1–53.

Allendorf, F.W., Gellman, W.A., Thorgaard, G.H., 1994. Sex-linkage of two enzyme loci in *Oncorhynchus mykiss* (rainbow trout). Heredity 72, 498–507.

Al-Marzouk, A., Lone, K.P., Teng, S.K., 1994. Photoperiod and temperature effects on the spawning time, fecundity and hatching success of a protandrous teleost, *Sparidentex hasta* Valenciennes. Pak. J. Zool. 26, 321–326.

Almeida-Toledo, L.F., Foresti, F., Daniel, M.F.Z., Toledo-Filho, S.A., 2000a. Sex chromosome evolution in fish: the formation of the neo-Y chromosome in Eigenmannia (Gymnotiformes). Chromosoma (Berlin) 109, 197–200.

Almeida-Toledo, L.F., Daniel-Silva, M.F.Z., Lopes, C.E., Toledo-Filho, S.d.A., 2000b. Sex chromosome evolution in fish: II. Second occurrence of an X1X2Y sex chromosome system in Gymnotiformes. Chromosome Res. 8, 335–340.

Alves, M.J., Coelho, M.M., Collares-Pereira, M.J., 1998. Diversity in the reproductive modes of females of the *Rutilus alburnoides* complex (Teleostei, Cyprinidae): a way to avoid the genetic constraints of uniparentalism. Mol. Biol. Evol. 15, 1233–1242.

Amador, L.M., 1982. Reproductive biology of the fairy basslet *Gramma loreto* Poey, M.S. Thesis, Department of Marine Science, University of Puerto Rico, 39 pp.

Amemiya, C.T., Gold, J.R., 1987. Karyology of 12 species of North American Cyprinidae (minnows) from the southern United States. Cytologia 52, 715–719.

Andersen, D., Boetius, I., Larsen, L.O., Seidler, P.H., 1996. Effects of oestradiol-enrich diet and of feeding with porcine testicular tissue on macroscopic gonadal sex in European eels. J. Fish Biol. 48, 484–492.

Andersson, T., 1990. Sex differences in cytochrome *P*-450-dependent xenobiotic and steroid metabolism in the mature rainbow trout kidney. J. Endocrinol. 126, 9–16.

Andersson, T., 1992. Purification, characterization and regulation of a male-specific cytochrome *P*450 in the rainbow trout kidney. Respir. Mar. Org. Pollut., 1–4, Part 34.

Andersson, T., Foerlin, L., 1992. Regulation of the cytochrome *P*450 enzyme system in fish. Aquat. Toxicol. 24, 1–20.

Anderson, W.D., Johnson, G.D., 1984. A new species of Callanthias (Pisces: Perciformes: Percoidei: Callanthii-

dae) from the southeastern Pacific Ocean, Proceedings of the Biological Society of Washington, Washington DC vol. 97, pp. 942–950.

Anderson, C.E., Smitherman, R.O., Shelton, W.L., Grover, J.H., 1978. Production of normal male and androgen sex-reversed *Tilapia aurea* and *T. nilotica* fed a commercial catfish diet in ponds. Symposium on Culture of Exotic Fishes presented at Aquaculture/Atlanta/'78, Atlanta, Georgia, January.

Andreata, A.A., Almeida-Toledo, L.F., Oliveira, C., Toledo Filho, S.D.A., 1992. Chromosome studies in Hypoptopomatinae (Pisces, Siluriformes, Loricariidae): 1. XX/XY sex chromosome heteromorphism in *Pseudotocinclus tietensis*. Cytologia 57, 369–372.

Andreata, A.A., do Almeida-Toledo, L., Oliveira, C., Toledo Filho, S.d.A., 1993. Chromosome studies in hypoptopomatinae (Pisces, Siluriformes, Loricariidae): II. ZZ/ZW sex-chromosome system, B chromosomes, and constitutive heterochromatin differentiation in *Microlepidogaster leucofrenatus*. Cytogenet. Cell Genet. 63, 215–220.

Andrew, T.G., Buxton, C.D., Hecht, T., 1996. Aspects of the reproductive biology of the concha wrasse, *Nelabrichthys ornatus*, at Tristan da Cunha. Environ. Biol. Fishes 46, 139–149.

Angus, R.A., 1989. Inheritance of melanistic pigmentation in the eastern mosquitofish. J. Hered. 80, 387–392.

Aoyama, T., 1955. On the hermaphroditism in the yellow sea bream, *Taius tumifrons*. Jpn. J. Ichthyol. 4, 119–129.

Arai, K., 2001. Genetic improvement of aquaculture finfish species by chromosome manipulation techniques in Japan. Aquaculture 197, 205–228.

Arai, R., Inoue, M., 1976. Chromosomes of seven species of Pomacentridae and two species of Acanthuridae from Japan. Bull. Natl. Sci. Mus., Ser. A (Tokyo) 2, 74–78.

Arai, K., Mukaino, M., 1997. Clonal nature of gynogenetically induced progeny of triploid (diploid × tetraploid) loach, *Misgurnus anguillicaudatus* (Pisces: Cobitididae). J. Exp. Zool. 278, 412–421.

Arai, R., Sawada, Y., 1974. Chromosomes of Japanese gobioid fishes (I). Bull. Natl. Sci. Mus. (Tokyo) 17, 97–102.

Arai, R., Inoue, M., Ida, H., 1976. Chromosomes of four species of coral fishes from Japan. Bull. Natl. Sci. Mus., Ser. A (Tokyo) 2, 137–142.

Arai, K., Matsubara, K., Suzuki, R., 1993. Production of polyploids and viable gynogens using spontaneously occurring tetraploid loach, *Misgurnus anguillicaudatus*. Aquaculture 117, 227–235.

Arai, K., Taniura, K., Zhang, Q., 1999. Production of second generation progeny of hexaploid loach. Fish. Sci. 65, 186–192.

Araki, K., Shinma, H., Nagoya, H., Nakayama, I., Onozato, H., 1995. Androgenetic diploids of rainbow trout (*Oncorhynchus mykiss*) produced by fused sperm. Can. J. Fish. Aquat. Sci. 52, 892–896.

Arefjev, V.A., 1989. Karyotype variability in successive generations after hybridization between the great sturgeon *Huso huso* L. and the sterlet *Acipenser ruthenus* L. J. Fish Biol. 35, 819–828.

Arkhipchuk, V.V., 1995. Role of chromosomal and genome mutations in the evolution of bony fishes. Hydrobiol. J. 31, 55–65.

Artoni, R.F., Venere, P.C., Bertollo, L.A.C., 1998. A heteromorphic ZZ/ZW sex chromosome system in fish, genus *Hypostomus* (Loricariidae). Cytologia (Tokyo) 63, 421–425.

Artoni, R.F., Falcao, J.D.N., Moreira-Filho, O., Bertollo, L.A.C., 2001. An uncommon condition for a sex chromosome system in Characidae fish: distribution and differentiation of the ZZ/ZW system in *Triportheus*. Chromosome Res. 9, 449–456.

Arukwe, A., Knudsen, F.R., Goksoyr, A., 1997. Fish zona radiata (eggshell) protein: a sensitive biomarker for environmental estrogens. Environ. Health Persp. 105, 418–422.

Asahina, K., Aida, K., Higashi, T., 1993. Biosynthesis of 17 alpha, 20 alpha-dihydroxy-4-pregnen-3-one from 17 alpha-hydroxyprogesterone by goldfish (*Carassius auratus*) spermatozoa. Zool. Sci. 10, 381–383.

Ashfield, L.A., Pottinger, T.G., Sumpter, J.P., 1998. Exposure of female juvenile rainbow trout to alkylphenolic compounds results in modifications to growth and ovosomatic index. Environ. Toxicol. Chem. 17, 679–686.

Asoh, K., Shapiro, D.Y., 1997. Bisexual juvenile gonad and gonochorism in the fairy basslet, *Gramma loreto*. Copeia 1997, 22–31.

Atz, J.W., 1964. Intersexuality in fishes. In: Armstrong, C.N., Marshall, A.J. (Eds.), Intersexuality in Vertebrates Including Man. Academic Press, London, pp. 145–232.

Avise, J.C., Trexler, J.C., Travis, J., Nelson, W.S., 1991. *Poecilia mexicana* is the recent female parent of the unisexual fish *P. formosa*. Evolution 45, 1530–1533.

Avtalion, R.R., Don, J., 1990. Sex-determining genes in tilapia a model of genetic recombination emerging from sex ratio results of three generations of diploid gynogenetic *Oreochromis aureus*. J. Fish Biol. 37, 167–174.

Avtalion, R.R., Hammerman, I.S., 1978. Sex determinaton in Sarotherodon (Tilapia): 1. Introduction to a theory of autosomal influence. Bamidgeh 30, 110–115.

Badcock, J., 1986. Aspects of the reproductive biology of *Gonostoma bathyphilum* (Gonostomatidae). J. Fish Biol. 29, 589–603.

Badura, L.L., Friedman, H., 1988. Sex reversal in female *Betta splendens* as a function of testosterone manipulation and social influence. J. Comp. Psychol. 102, 262–268.

Baker, I., Solar, I., Donaldson, E., 1988. Masculinization of chinook salmon (*Oncorhynchus tshawytscha*) by immersion treatments using 17 alpha-methyltestosterone around the time of hatching. Aquaculture 72, 359–367.

Balinsky, B.I., 1975. An introduction to Embryology. Saunders, Philadelphia, 648 pp.

Ball, S.E., Forrester, L.M., Wolf, C.R., Back, D.J., 1990. Differences in the cytochrome *P*-450 isozymes involved in the 2-hydroxylation of oestradiol and 17alpha-ethynylestradiol. Biochem. J. 262, 221–226.

Bang, I.C., Kim, Y., Kim, K.K., Lee, J.K., 1995. Studies on the production of all-female populations of olive flounder, *Paralichthys olivaceus*: 2. Hormonal sex reversal. Bull. Natl. Fish. Res. Dev. Agency 49, 49–57.

Baras, E., Prignon, C., Gohoungo, G., Melard, C., 2000. Phenotypic sex differentiation of blue tilapia under constant and fluctuating thermal regimes and its adaptive and evolutionary implications. J. Fish Biol. 57, 210–223.

Baras, E., Jacobs, B., Melard, C., 2001. Effect of water temperature on survival, growth and phenotypic sex of mixed (XX–XY) progenies of Nile tilapia *Oreochromis niloticus*. Aquaculture 192, 187–199.

Baroiller, J.F., D'Cotta, H., 2000. Environment and sex determination in farmed fish. Comp. Biochem. Physiol., A, S10.

Baroiller, J.F., Chourrout, D., Fostier, A., Jalabert, B., 1995. Temperature and sex chromosomes govern sex ratios of the mouthbrooding cichlid fish *Oreochromis niloticus*. J. Exp. Zool. 273, 216–223.

Baroiller, J.F., Fostier, A., Cauty, C., Rognon, X., Jalabert, B., 1996. Effects of high rearing temperatures on the sex ratio of progeny from sex reversed males of *Oreochromis niloticus*. The Third International Symposium on Tilapia in Aquaculture. ICLARM, Makati City, p. 41.

Baroillier, J.-F., Guigen, Y., Fostier, A., 1999. Endocrine and environmental aspects of sex differentiation in fish. Cell. Mol. Life Sci. 55, 910–931.

Barry, T.P., Aida, K., Okumura, T., Hanyu, I., 1990. The shift from C-19 to C-21 steroid synthesis in spawning male common carp, *Cyprinus carpio*, is regulated by the inhibition of androgen production by progestogens produced by spermatozoa. Biol. Reprod. 43, 105–112.

Basavaraja, N., Nandeesha, M.C., Varghese, T.J., Keshavanath, P., Srikanth, G.K., 1990. Induction of sex reversal in *Oreochromis mossambicus* by diethylstilbestrol. J. Appl. Ichthyol. 6, 46–50.

Basavaraja, N., Gangadhar, B., Udupa, K.S., 1997. Effect of diethylstilbestrol-incorporated diet on sex ratio and body composition of common carp, *Cyprinus carpio*. J. Aquacult. Trop. 12, 209–218.

Basolo, A.L., 1994. The dynamics of fisherian sex-ratio evolution: theoretical and experimental investigations. Am. Nat. 144, 473–490.

Basolo, A.L., 2001. The effect of intrasexual fitness differences on genotype frequency stability at fisherian sex ratio equilibrium. Ann. Zool. Fenn. 38, 297–304.

Beacham, T.D., Murray, C.B., 1983. Sexual dimorphism in the adipose fin of Pacific salmon (*Oncorhynchus*). Can. J. Fish. Aquat. Sci. 40, 2019–2024.

Beacham, T.D., Murray, C.B., 1986. Sexual dimorphism in length of upper jaw and adipose fin of immature and maturing Pacific salmon (*Oncorhynchus*). Aquaculture 58, 3–4.

Beamish, F.W.H., 1993. Environmental sex determination in southern brook lamprey, *Ichthyomyzon gagei*. Can. J. Fish. Aquat. Sci. 50, 1299–1307.

Beardmore, J.A., Mair, G.C., Lewis, R.I., 2001. Monosex male production in finfish as exemplified by tilapia: applications, problems, and prospects. Aquaculture 197, 283–301.

Bebars, M., 1978. *Scarus ghardaqensis*, n.sp., a new parrotfish (Pisces, Scaridae) from the Red Sea, with a note on sexual dichromatism in the family. Cybium 3e ser., 76–81.

Becak, W., Becak, M.L., Ohno, S., 1966. Cited in Rishi, K.K. Current status of fish cytogenetics. In: Das and Jhingram (Eds.), Fish Genetics in India. Cytogenetics. vol. 5. Today and Tomorrow's Printers, New Delhi, India, p. 315.

Begay, V., Valotaire, Y., Ravault, J.P., Collin, J.P., Falcon, J., 1994. Detection of estrogen receptor mRNA in trout pineal and retina: estradiol-17-beta modulates melatonin production by cultured pineal photoreceptor cells. Gen. Comp. Endocrinol. 93, 61–69.

Bellamy, A.W., 1936. Interspecific hybrids in *Platypoecilus*: one species ZZ–ZW; the other XY–XX. Proc. Natl. Acad. Sci. U. S. A. 22, 531–536.

Bende, C., 1982. Study on the methyltestosterone induction of sex reversal in gynogenetic progeny of crucian carp. J. Fish. China 6, 147–152.

Benfey, T.J., 1999. The physiology and behaviour of triploid fish. Rev. Fish. Sci. 7, 39–67.

Benfey, T., Solar, I., de Jong, G., Donaldson, E., 1986. Flow-cytometric confirmation of aneuploidy in sperm from triploid rainbow trout. Trans. Am. Fish. Soc. 115, 838–840.

Benfey, T.J., Dye, H.E., Donaldson, E.M., 1989. Estrogen-induced vitellogenin production by triploid coho salmon (*Oncorhynchus kisutch*), and its effect on plasma and pituitary gonadotropin. Gen. Comp. Endocrinol. 75, 83–87.

Bennett, D., Goodyear, C., 1978. Response of mosquitofish to thermal effluent. Energy and Environmental Stress in Aquatic Systems. Technical Information Center.

Bentivegna, F.B.F.B.A.M.A., 1989. Some aspects of reproduction of *Symphodus ocellatus*. Oebalia (Taranto) 15, 909–911.

Bentivegna, F., Rasotto, M.B., 1987. Protogynous hermaphroditism in *Xyrichthys novacula* (L. 1758). Cybium Ser. 3 (11), 75–78.

Bentivegna, F., Cirino, P., Rasotto, M.B., 1985. Further investigations into sex reversal of *Coris julis* L. (Pisces, Labridae). Boll. Zool. 52, 3–4.

Bertollo, L.A.C., Cavallaro, Z.I., 1992. A highly differentiated ZZ/ZW sex chromosome system in a Characidae fish, *Triportheus guentheri*. Cytogenet. Cell Genet. 60, 60–63.

Bertollo, L.A., Mestriner, C.A., 1998. The X1X2Y sex chromosome system in the fish *Hoplias malabaricus*: II. Meiotic analyses. Chromosome Res. 6, 141–147.

Bertollo, L.A.C., Takahashi, C.S., Moreira-Filho, O., 1978. Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae). Rev. Bras. Genet. 1, 103–120.

Bertollo, L.A.C., Takahashi, C.S., Filho, O.M., 1983. Multiple sex chromosomes in the genus *Hoplias* (Pisces: Erythrinidae). Cytologia 48, 1–12.

Bertollo, L.A.C., Born, G.G., Dergam, J.A., Fenocchio, A.S., Moreira-Filho, O., 2000. A biodiversity approach in the neotropical Erythrinidae fish, *Hoplias malabaricus*: karyotypic survey, geographic distribution of cytotypes and cytotaxonomic considerations. Chromosome Res. 8, 603–613.

Besseau, L., Faliex, E., 1989. Presence of granulocytes and brown bodies in the ovotestis of *Lithognathus mormyrus* (L.) (Teleost, Sparidae). Ichtyophysiol. Acta, 109–114.

Beullens, K., Eding, E.H., Gilson, P., Ollevier, F., Komen, J., Richter, C.J.J., 1997. Gonadal differentiation, intersexuality and sex ratios of European eel (*Anguilla anguilla* L.) maintained in captivity. Aquaculture 153, 135–150.

Bhattacharya, S., Halder, S., Manna, P.R., 1994. Current status of endocrine aspects of fish production: an overview. Proc. Indian Natl. Sci. Acad., Part B 60, 33–43.

Bieniarz, K., 1986. Sex differentiation and puberty in cyprinids. Aquacult. Cyprinids, Aquacult. Cyprinides Colloq. Hydrobiol., 101–108.

Bieniarz, K., Epler, P., 1992. Advances in reproductive endocrinology of fish. J. Physiol. Pharmacol. 43, 215–222.

Bieniarz, K., Epler, P., Malczewski, B., Passakas, T., 1981. Development of European eel (*Anguilla anguilla* L.) gonads in artificial conditions. Aquaculture 22, 53–66.

Bieniarz, K., Goryczko, K., Dobosz, S., Grudniewski, T., 1991. The effects of 17 methyltestosterone on rainbow trout *Oncorhynchus mykiss*. Pol. Arch. Hydrobiol. 38, 295–301.

Bieniarz, K., Koldras, M., Mejza, T., 1997. Unisex and polyploid populations of cyprinid and silurid fish. Arch. Ryb. Pol./Arch. Pol. Fish. 5, 31–36.

Billard, R., Richard, M., Rombauts, R., 1982. Inhibition of spermatogenesis and vitellogenesis in rainbow trout by hormonal additives in the diet. Prog. Fish-Cult. 44, 15–18.

Black, D.A., Howell, W.M., 1979. The North American mosquitofish, *Gambusia affinis*: a unique case in sex chromosome evolution. Copeia 1979, 509–513.

Blanc, J.M., Poisson, H., Escaffre, A.M., Aguirre, P., Vallee, F., 1993. Inheritance of fertilizing ability in male tetraploid rainbow trout (*Oncorhynchus mykiss*). Aquaculture 110, 61–70.

Blázquez, M., Piferrer, F., Zanuy, S., Carrillo, M., Donaldson, E.M., 1995. Development of sex control techniques for European sea bass (*Dicentrarchus labrax* L.) aquaculture: effects of dietary 17 alpha-methyltestosterone prior to sex differentiation. Aquaculture 135, 329–342.

Blázquez, M., Zanuy, S., Carillo, M., Piferrer, F., 1998a. Structural and functional effects of early exposure to estradiol-17 beta and 17 alpha-ethynylestradiol on the gonads of the gonochoristic teleost *Dicentrarchus labrax*. Fish Physiol. Biochem. 18, 37–47.

Blázquez, M., Zanuy, S., Carillo, M., Piferrer, F., 1998b. Effects of rearing temperature on sex differentiation in the European sea bass (*Dicentrarchus labrax* L.). J. Exp. Zool. 281, 207–216.

Blázquez, M., Carrillo, M., Zanuy, S., Piferrer, F., 1999. Sex ratios in offspring of sex-reversed sea bass and the relationship between growth and phenotypic sex differentiation. J. Fish Biol. 55, 916–930.

Blázquez, M., Felip, A., Zanuy, S., Carrillo, M., Piferrer, F., 2001. Critical period of androgen-inducible sex differentiation in a teleost fish, the European sea bass. J. Fish Biol. 58, 342–358.

Bolla, S., 1987. Cytogenetic studies in Atlantic salmon and rainbow trout embryos. Hereditas 106, 11–17.

Bon, E., Barbe, U., Nunez Rodriguez, J., Cuisset, B., Pelissero, C., Sumpter, J.P., Le Menn, F., 1997. Plasma vitellogenin levels during the annual reproductive cycle of the female rainbow trout (*Oncorhynchus mykiss*): establishment and validation of an ELISA. Comp. Biochem. Physiol., B 117B, 75–84.

Boney, S.E., Shelton, W.L., Yang, S.L., Wilken, L.O., 1984. Sex reversal and breeding of grass carp. Trans. Am. Fish. Soc. 113, 348–353.

Bongers, A.B.J., in't Veld, E.P.C., Abo-Hashema, K., Bremmer, I.M., Eding, E.H., Komen, J., Richter, C.J.J., 1994. Androgenesis in common carp (*Cyprinus carpio* L.) using UV irradiation in a synthetic ovarian fluid and heat shocks. Aquaculture 122, 2–3.

Bongers, A.B.J., Zandieh-Doulabi, B., Richter, C.J.J., Komen, J., 1999. Viable androgenetic YY genotypes of common carp (*Cyprinus carpio* L.). J. Hered. 90, 195–198.

Borg, B., Timmers, R.J.M., Lambert, J.G.D., 1987. Aromatase activity in the brain of the three-spined stickleback, *Gasterosteus aculeatus*: 1. Distribution and effects of season and photoperiod. Exp. Biol. 47, 63–68.

Borg, B., Mayer, I., Lambert, J., Granneman, J., Schulz, R., 1992. Metabolism of androstenedione and 11-ketotestosterone in the kidney of the three-spined stickleback, *Gasterosteus aculeatus*. Gen. Comp. Endocrinol. 86, 248–256.

Borg, B., Antonopoulou, E., Mayer, I., Andersson, E., Berglund, I., Swanson, P., 1998. Effects of gonadectomy and androgen treatments on pituitary and plasma levels of gonadotropins in mature male Atlantic salmon, *Salmo salar*, parr-positive feedback control of both gonadotropins. Biol. Reprod. 58, 814–820.

Born, G.G., Bertollo, L.A.C., 2000. Comparative cytogenetics among allopatric populations of the fish, *Hoplias malabaricus*: cytotypes with $2n=42$ chromosomes. Genetica (Dordrecht) 110, 1–9.

Boron, A., 1994. Karyotypes of diploid and triploid silver crucian carp *Carassius auratus* gibelio (Bloch). Cytobios 80, 117–124.

Boron, A., 1995. Chromosome banding studies of spined loach *Cobitis taenia* (L.). Cytobios 81, 97–102.

Borquez, R.S., Olivares, P.A., Tapia, M.L., 1988. Gonadic structure and sexual inversion in "cabrilla comun" *Paralabrax humeralis* Valenciennes, 1828 (Pisces, Serranidae). Estud. Oceanol. Inst. Invest. Oceanol. Univ. Antofagasta 7, 51–58.

Bortone, S.A., Davis, W.P., 1994. Fish intersexuality as indicator of environmental stress. Bioscience 44, 165–172.

Breitburg, D.L., 1987. Interspecific competition and the abundance of nest sites: factors affecting sexual selection. Ecology 68, 1844–1855.

Brennan, J., Karl, J., Martineau, J., Nordqvist, K., Schmahl, J., Tilmann, C., Ung, K., Capel, B., 1998. Sry and the testis: molecular pathways of organogenesis. J. Exp. Zool. 281, 494–500.

Bridges, C.B., 1925. Sex in relation to chromosomes and genes. Am. Nat. 59, 127–137.

Brooks, S., Tyler, C.R., Sumpter, J.P., 1997. Egg quality in fish: what makes a good egg? Rev. Fish Biol. Fish. 7, 387–416.

Bruce, R.W., 1980. Protogynous hermaphroditism in two marine angelfishes. Copeia 1980, 353–355.

Brum, M.J.I., 1996. Cytogenetic studies of Brazilian marine fish. Braz. J. Genet. 19, 421–427.
Brunner, B., Hornung, U., Shan, Z., Nanda, I., Kondo, M., Zend-Ajusch, E., Haaf, T., Ropers, H.-H., Shima, A., Schmid, M., Kalscheuer, V.M., Schartl, M., 2001. Genomic organization and expression of the doublesex-related gene cluster in vertebrates and detection of putative regulatory regions for DMRT1. Genomics 77, 8–17.
Bruslé, S., 1987. Sex-inversion of the hermaphroditic, protogynous teleost *Coris julis* L. (Labridae). J. Fish Biol. 30, 605–616.
Bruslé, S., 1988. Sex differentiation in teleosts: primordial germ cells as stem cells. Reproduction in fish: basic and applied aspects in endocrinology and genetics. Colloq. Inst. Natl. Rech. Agron. 44, 21–24.
Bruslé, J., Bruslé, S., 1976. Contribution to the study of the reproduction of two species of grouper (*Epinephelus aeneus* and *E. guaza*) from the coasts of Tunisia. Rapp. P.-V. Reun.-Comm. Int. Explor. Sci. Mer Mediterr. (Monaco) 23, 49–50.
Bruslé, S., Bruslé, J., 1978a. An ultrastructural study of early germ cells in *Mugil* (Liza) *auratus* Risso, 1810 (Teleostei: Mugilidae). Ann. Biol. Anim. Biochim. Biophys., 1141–1153.
Bruslé, S., Bruslé, J., 1978b. Early sex differentiation in *Mugil* (Liza) *auratus* Risso, 1810 (Teleost Mugilidae). An ultrastructural study. Ann. Biol. Anim. Biochim. Biophys. 18, 871–875.
Bruslé, S., Debas, L., Cauty, C., 1989. Morphological and cytological aspects of sex inversion in a protogynous hermaphrodite, *Epinephelus microdon* (Teleostei, Serranidae), Advances In Tropical Aquaculture, Tahiti, French Polynesia. Actes Colloq. IFREMER, Tahiti, French Polynesia.
Bruslé Sicard, S., Fourcault, B., 1997. Recognition of sex-inverting protandric *Sparus aurata*: ultrastructural aspects. J. Fish Biol. 50, 1094–1103.
Bruslé Sicard, S., Reinboth, R., 1990. Protandric hermaphrodite peculiarities in *Amphiprion frenatus* Brevoort Teleostei Pomacentridae. J. Fish Biol. 36, 383–390.
Bruslé Sicard, S., Debas, L., Fourcault, B., Fuchs, J., 1992. Ultrastructural study of sex inversion in a protogynous hermaphrodite *Epinephelus microdon* Teleostei Serranidae. Reprod. Nutr. Dev. 32, 393–406.
Bruslé Sicard, S., Reinboth, R., Fourcault, B., 1994. Germinal potentialities during sexual state changes in a protandric hermaphrodite, *Amphiprion frenatus* (Teleostei, Pomacentridae). J. Fish Biol. 45, 597–611.
Bull, J.J., 1983. Evolution of Sex Determining Mechanisms Benjamin/Cummings, Menlo Park, CA, 316 pp.
Bull, J.J., 1985. Sex determining mechanisms: an evolutionary perspective. Experientia 41, 1285–1296.
Bull, J.J., Vogt, R.C., 1979. Temperature-dependent sex determination in turtles. Science 206, 1186–1188.
Bullock, L.H., Murphy, M.D., 1994. Aspects of the life history of the yellowmouth grouper, *Mycteroperca interstitialis*, in the eastern Gulf of Mexico. Bull. Mar. Sci. 55, 30–45.
Buxton, C.D., 1990. The reproductive biology of *Chrysoblephus laticeps* and *C. cristiceps* (Teleostei: Sparidae). J. Zool. 220, 497–511.
Buxton, C.D., Clarke, J.R., 1986. Age, growth and feeding of the blue hottentot *Pachymetopon aeneum* (Pisces: Sparidae) with notes on reproductive biology. S. Afr. J. Zool. 21, 33–38.
Buxton, C.D., Garratt, P.A., 1990. Alternative reproductive styles in seabreams (Pisces: Sparidae). Environ. Biol. Fishes 28, 113–124.
Bye, V., Lincoln, R., 1981. Get rid of the males and let the females prosper. Fish Farmer 4, 22–24.
Calhoun, W.E., Shelton, W.L., 1983. Sex ratios of progeny from mass spawnings of sex-reversed broodstock of *Tilapia nilotica*. Aquaculture 33, 1–4.
Callard, G.V., Tchoudakova, A., 1997. Evolutionary and functional significance of two CYP19 genes differentially expressed in brain and ovary of goldfish. J. Steroid Biochem. 61, 387–392.
Callard, G.V., Manz, L., Petro, Z., Claiborne, J.B., 1982. Brain estrogen biosynthesis and estrogen conjugating systems in the sculpin (*Myoxocephalus*). Bull.-Mt. Desert Isl. Biol. Lab. 22, 41–43.
Calvo, J., Morriconi, E., Rae, G.A., San Roman, N.A., 1992. Evidence of protandry in subantarctic notothenid *Eleginops maclovinus* Cuv. and Val. 1830 from the beagle channel Argentina. J. Fish Biol. 40, 157–164.
Campbell, P.M., Devlin, R.H., 1996. Expression of CYP1A1 in livers and gonads of *Pacific salmon*: quantitation of mRNA levels by RT-cPCR. Aquat. Toxicol. 34, 47–69.
Campbell, P.M., Pottinger, T.G., Sumpter, J.P., 1994. Preliminary evidence that chronic confinement stress reduces the quality of gametes produced by brown and rainbow trout. Aquaculture 120, 1–2.
Campos, H.H., 1972. Karyology of three galaxiid fishes *Galaxias maculatus*, *G. platei*, and *Brachygalaxias bullocki*. Copeia 1972, 368–370.

Campos, H.H., Hubbs, C., 1971. Cytomorphology of six species of gambusiine fishes. Copeia 1971, 161–163.

Campos-Ramos, R. et al., 2001. Identification of putative sex chromosomes in the blue tilapia, *Oreochromis aureus*, through synaptonemal complex and FISH analysis. Genetica 111, 143–153.

Canario, A.V.M., Scott, A.P., 1989. Synthesis of 20 alpha-hydroxylated steroids by ovaries of the dab (*Limanda limanda*). Gen. Comp. Endocrinol. 76, 147–158.

Canario, A.V.M., Scott, A.P., 1990. Effects of steroids and human chorionic gonadotrophin on in vitro oocyte final maturation in two marine flatfish: The Dab, *Limanda limanda*, and the Plaice, *Pleuronectes platessa*. Gen. Comp. Endocrinol. 77, 161–176.

Cano, J., Pretel, A., Melendez, S., Garcia, F., Caputo, V., Fenocchio, A.S., Bertollo, L.A.C., 1996. Determination of early stages of sex chromosome differentiation in the sea bass *Dicentrarchus labrax* L. (Pisces: Perciformes). Cytobios 87, 45–59.

Capel, B., 1996. The role of Sry in cellular events underlying mammalian sex determination. Curr. Top. Dev. Biol. 32, 1–37.

Capel, B., 1998. Sex in the 90s: SRY and the switch to the male pathway. Annu. Rev. Physiol. 60, 497–523.

Capriglione, T., Morescalchi, A., Olmo, E., Rocco, L., Stingo, V., Manzo, S., 1994. Satellite DNAs, heterochromatin and sex chromosomes in *Chiondraco hamatus* (Channichthyidae, Perciformes). Polar Biol. 14, 285–290.

Caputo, V., Marchegiani, F., Olmo, E., 1996a. Karyotype differentiation between two species of carangid fishes, genus *Trachurus* (Perciformes: Carangidae). Mar. Biol. 127, 193–199.

Caputo, V., Vitturi, R., Odierna, G., Cano, J., Olmo, E., Colomba, M.S., 1996b. Characterization of mitotic chromosomes in the Gobiid fish *Zosterisessor ophiocephalus* (Pallas, 1811) (Perciformes, Gobiidae). Biol. Zentralbl. 115, 328–336.

Caputo, V., Machella, N., Nisi-Cerioni, P., Olmo, E., 2001. Cytogenetics of nine species of Mediterranean blennies and additional evidence for an unusual multiple sex-chromosome system in *Parablennius tentacularis* (Perciformes, Blenniidae). Chromosome Res. 9, 3–12.

Carbone, P., Vitturi, R., Catalano, E., Macaluso, M., 1987. Chromosome sex determination and Y-autosome fusion in *Blennius tentacularis* Brunnich, 1765 (Pisces, Blennidae). J. Fish Biol. 31, 597–602.

Cardwell, J.R., 1990. Behavioural endocrinology of the stoplight parrotfish, *Sparisoma viride*, Scaridae, a protogynous coral reef fish. Dissert. Abstr. Int. B 50, 12.

Cardwell, J.R., Liley, N.R., 1991. Hormonal control of sex and color change in the stoplight parrotfish, *Sparisoma viride*. Gen. Comp. Endocrinol. 81, 7–20.

Carrasco, L.A.P., Penman, D.J., Bromage, N., 1999. Evidence for the presence of sex chromosomes in the nile tilapia (*Oreochromis niloticus*) from synaptonemal complex analysis of XX, XY, and YY genotypes. Aquaculture 173, 207–218.

Carrozzo, R., Ellison, J., Yen, P., Taillon-Miller, P., Brownstein, B.H., Perisco, G., Ballabio, A., Shapiro, L., 1992. Isolation and characterization of a yeast artificial chromosome (YAC) contig around the human steroid sulfatase gene. Genomics 12, 7–12.

Carruth, L.L., 2000. Freshwater Cichlid *Crenicara punctulata* is a Protogynous Sequential Hermaphrodite. Copeia 2000, 71–82.

Carvalho, E.D., Foresti, F., 1996. Sex reversal in Nile tilapia, *Oreochromis niloticus* Trewavas, 1983, induced by 17-alpha-methyltestosterone: sex ratio and histological studies on the gonads. Rev. Bras. Biol. 56, 249–262.

Castelli, M., Philippart, J.C., 1993. Sex determination in the genus *Barbus* (Osteichthyes, Cyprinidae). Proceedings of the International Round Table *Barbus*. Cah. Ethol. Fondam. Appl. Anim. Hum., Univ. Liege, pp. 191–194.

Cataudella, S., Civitelli, M.V., Capanna, E., 1973. The chromosomes of some Mediterranean teleosts: Scorpaenidae, Serranidae, Labridae, Blennidae, Gobiidae (Pisces, Scorpaeniformes, Perciformes). Boll. Zool. 40, 385–389.

Cau, A., Coluccia, E., Deiana, A.M., Pichiri, G., Rossino, R., Salvadori, S., Mezzanotte, R., 1992. Chromosomes and DNA of *Anguilla anguilla*: a study with restriction endonucleases. Genome 35, 838–843.

Cavaco, J.E.B., Lambert, J.G.D., Schulz, R.W., Goos, H.J.T., 1997. Pubertal development of male African catfish, *Clarias gariepinus*: in vitro steroidogenesis by testis and interrenal tissue and plasma levels of sexual steroids. Fish Physiol. Biochem. 16, 129–138.

Chakraborty, S.S.T.P., 1993. Effect of endosulfan (thiodan) on vitellogenesis and its modulation by different hormones in the vitellogenic catfish *Clarias batrachus*. Respir. Mar. Org. Pollut. 35, 225–226.

Chan, S.T.H., Yeung, W.S.B., 1983. Sex Control and Sex Reversal in Fish Under Natural Conditions. Academic Press, New York, pp. 171–222.

Chan, S.T.H., Yeung, W.S.B., 1989. Sex steroids in intersexual fishes. Proc. First Int. Symp. Fish 7, 1–6.

Chang, C.F., Lin, B.Y., 1998. Estradiol-17-beta stimulates aromatase activity and reversible sex change in protandrous black porgy, *Acanthopagrus schlegeli*. J. Exp. Zool. 280, 165–173.

Chang, J.P., Peter, R.E., 1983a. Effects of dopamine on gonadotropin release in female goldfish, *Carassius auratus*. Neuroendocrinology 36, 351–357.

Chang, J.P., Peter, R.E., 1983b. Effects of pimozide and des Gly super(10), (D-Ala super(6)) luteinizing hormone-releasing hormone ethylamide on serum gonadotropin concentrations, germinal vesicle migration, and ovulation in female goldfish, *Carassius auratus*). Gen. Comp. Endocrinol. 52, 30–37.

Chang, C.F., Yueh, W.S., 1990a. Oocyte maturation in protandrous black porgy *Acanthopagrus schlegeli* stimulated by enclomiphene and Lhrh analogue. Bull. Inst. Zool. Acad. Sin. 29, 173–180.

Chang, C.F., Yueh, W.S., 1990b. Annual cycle of gonadal histology and steroid profiles in the juvenile males and adult females of the protandrous black porgy *Acanthopagrus schlegeli*. Aquaculture 91, 179–196.

Chang, C.F., Yueh, W.S., Lee, M.F., 1991. Effects of LHRH-A and HCG on the steroid profiles of bisexual and mature male and female protandrous black porgy, *Acanthopagrus schlegeli*. Aquaculture 92, 83–92.

Chang, J.P., Jobin, R.M., Wong, A.O.L., 1993. Intracellular mechanisms mediating gonadotropin and growth hormone release in the goldfish, *Carassius auratus*. Fish Physiol. Biochem. 11, 25–33.

Chang, C.F., Lee, M.F., Chen, G.R., 1994. Estradiol-17-beta associated with the sex reversal in protandrous black porgy, *Acanthopagrus schlegeli*. J. Exp. Zool. 268, 53–58.

Chang, C.F., Lau, E.L., Lin, B.Y., 1995a. The effects of exogenous estradiol-17 beta on gonadal development in juvenile males of protandrous black porgy, *Acanthopagrus schlegeli*. Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish, Fish Symposium 95, Austin, TX (USA), pp. 362.

Chang, C.F., Lau, E.L., Lin, B.Y., 1995b. Estradiol-17-beta suppresses testicular development and stimulates sex reversal in protandrous black porgy, *Acanthopagrus schlegeli*. Fish Physiol. Biochem. 14, 481–488.

Chang, C.-F., Lan, S.-C., Pan, B.S., 1995c. Feed administration of estradiol-17 beta stimulates female differentiation in juvenile grey mullet *Mugil cephalus*. Zool. Stud. 34, 257–264.

Chang, X.T., Kobayashi, T., Kajiura, H., Nakamura, M., Nagahama, Y., 1997. Isolation and characterization of the cDNA encoding tilapia (*Oreochromis niloticus*) cytochrome *P*450 aromatase (*P*450arom): changes in *P*450arom mRNA, protein, and enzyme activity in ovarian follicles during oogenesis. J. Mol. Endocrinol. 18, 57–66.

Chang, C.-F., Hung, C.-Y., Chiang, M.-C., Lan, S.-C., 1999a. The concentrations of plasma sex steroids and gonadal aromatase during controlled sex differentiation in grey mullet, *Mugil cephalus*. Aquaculture 177, 37–45.

Chang, X.T., Kobayashi, T., Todo, T., Ikeuchi, T., Yoshiura, Y., Kajiura-Kobayashi, H., Morrey, C., Nagahama, Y., 1999b. Molecular cloning of estrogen receptors alpha and beta in the ovary of a teleost fish, the tilapia (*Oreochromis niloticus*). Zool. Sci. 16, 653–658.

Charlesworth, B., 1991. The evolution of sex chromosomes. Science 251, 1030–1033.

Charlesworth, B., 1996. The evolution of chromosomal sex determination and dosage compensation. Curr. Biol. 6, 149–162.

Chauvet, C., 1988. Study of the growth of the grouper *Epinephelus guaza* (Linnaeus, 1758) from the Tunisian coast. Aquat. Living Resour. 1, 277–288.

Chen, T.R., 1969. Karyological heterogamety of deep-sea fishes. Postilla 130, 1–29.

Chen, T.R., Ebeling, A.W., 1968. Karyological evidence of female heterogamety in the mosquitofish, *Gambusia affinis*. Copeia 1968, 70–75.

Chen, T.R., Reisman, H.M., 1970. A comparative chromosome study of North American species of sticklebacks (Teleostei: Gasterostidae). Cytogenetics 9, 321–332.

Chen, T.R., Ruddle, F.H., 1970. A chromosome study of four species and a hybrid of the killifish geneus *Fundulus* (Cyrinodontidae). Chromosoma 29, 255–267.

Chen, T.T., Sonstegard, R.A., 1984. Development of a rapid, sensitive and quantitative test for the assessment of the effects of xenobiotics on reproduction in fish. Respir. Mar. Org. Pollut. 14, 1–4.

Chen, C.P., Hsieh, H.L., Chang, K.H., 1980. Some aspects of the sex change and reproductive biology of the grouper, *Epinephelus diacanthus* (Cuvier et Valenciensis). Bull. Inst. Zool., Acad. Sin. 19, 11–17.

Chen, M., Yang, X., Yu, X., Chen, H., 1996. Karyotype studies on the bisexual natural gynogenetic crucian carp (*Carassius auratus*) of Pengze. Acta Hydrobiol. Sin. 20, 25–31.

Cherfas, N.B., 1966. Natural triploidy in the females of the unisexual variety of the goldfish *Carassius auratus* gibelio (Bloch). Sov. Genet. 13, 557–563.

Cherfas, N.B., Rothbard, S., Hulata, G., Kozinsky, O., 1991. Spontaneous diploidization of maternal chromosome set in ornamental (koi) carp, *Cyprinus carpio* L. J. Appl. Ichthyol. 7, 72–77.

Cherfas, N.B., Gomelsky, B.I., Emelyanova, O.V., Recoubratsky, A.V., 1994. Induced diploid gynogenesis and polyploidy in crucian carp, *Carassius auratus* gibelio (Bloch), X common carp, *Cyprinus carpio* L., hybrids. Aquacult. Fish. Manage. 25, 943–954.

Chernenko, Y.V., 1976. Genome mutations among embryos of the diadromous and resident sockeye, *Oncorhynchus nerka*, from Lake Dal'neye (Kamchatka). J. Ichthyol. 16, 373–379.

Chevassus, B., 1983. Hybridization in fish. Aquaculture 33, 1–4.

Chevassus, B., Devaux, A., Chourrout, D., Jalabert, B., 1988. Production of YY rainbow trout males by self-fertilization of induced hermaphrodites. J. Hered. 79, 89–92.

Chiba, H., Iwatsuki, K., Hayami, K., Yamauchi, K., 1993. Effects of dietary estradiol-17-beta on feminization, growth and body composition in the Japanese eel (*Anguilla japonica*). Comp. Biochem. Physiol., A 106, 367–371.

Choat, J.H., Axe, L.M., Lou, D.C., 1996. Growth and longevity in fishes of the family Scaridae. Mar. Ecol.: Prog. Ser. (Oldendorf) 145, 33–41.

Choudhury, R.C., Prasad, R., Charan Das, C., 1982. Karyological studies in five tetraodontiform fishes from the Indian Ocean. Copeia 1982, 728–732.

Chourrout, D., Nakayama, I., 1987. Chromosome studies of progenies of tetraploid female rainbow trout. Theor. Appl. Genet. 74, 687–692.

Chourrout, D., Quillet, E., 1982. Induced gynogenesis in the rainbow trout: sex and survival of progenies production of all-triploid populations. Theor. Appl. Genet. 63, 201–205.

Chourrout, D., Chevassus, B., Krieg, F., Happe, A., Burger, G., Renard, P., 1986. Production of second generation triploid and tetraploid rainbow trout by mating tetraploid males and diploid females—potential of tetraploid fish. Theor. Appl. Genet. 72, 193–206.

Church, A., 1997. Ecology of the Norfolk Island domestic fishery. Diss. Abstr. Int., B 58, 1069.

Cimino, M.C., 1972a. Egg production, polyploidization and evolution in a diploid all-female fish of the genus *Poeciliopsis*. Evolution 26, 294–306.

Cimino, M.C., 1972b. Meiosis in triploid all-female fish (*Poeciliopsis*: Poeciliidae). Science 175, 1484–1485.

Clark, E., Shen, D., 1986. Territoriality of Red Sea sand-diving fishes of the genera *Xyrichtys* and *Trichonotus*. In: Uyeno, T., Arai, R., Taniuchi, T., Matsuura, K. (Eds.), Conference 2. Int. Conf. on Indo-Pacific Fishes, Tokyo (Japan).

Clark, E., Rabin, J.S., Holderman, S., 1988. Reproductive behavior and social organization in the sand tilefish, *Malacanthus plumieri*. Environ. Biol. Fishes 22, 273–286.

Clavijo, I.E., 1982. Distribution, reproductive biology, and social structure of the redband parrotfish, *Sparisoma aurofrenatum* (Valenciennes). Contrib. Dep. Mar. Sci. Univ. Puerto Rico 20, 151.

Clavijo, I.E., 1983. Sex change in the redband parrotfish. Proc. Assoc. Isl. Mar. Lab. Caribb. 17, 9.

Clemens, H., Inslee, T., 1968. The production of unisexual broods of *Tilapia mossambica* sex-reversed with methyltestodterone. Trans. Am. Fish. Soc. 97, 18–21.

Clemens, H., McDermitt, C., Inslee, T., 1966. The effects of feeding methyltestosterone to guppies for 60 days after birth. Copeia 1966, 280–284.

Clifton, D.R., Rodriguez, R.J., 1997. Characterization and application of a quantitative DNA marker that discriminates sex in chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 54, 2647–2652.

Cline, T.W., Meyers, B.J., 1996. Vive la difference: males vs. females in flies vs. worms. Annu. Rev. Genet. 30, 637–702.

Coates, D., 1982. Some observations on the sexuality of humbug damselfish, *Dascyllus aruanus* (Pisces, Pomacentridae) in the field. Z. Tierpsychol. 59, 7–18.

Cochran, R.C., Grier, H.J., 1991. Regulation of sexual succession in the protogynous black sea bass *Centropristis striatus* Osteichthyes Serranidae. Gen. Comp. Endocrinol. 82, 69–77.
Cody, R.P., Bortone, S.A., 1992. An investigation of the reproductive mode of the pinfish *Lagodon rhomboides* Linnaeus Osteichthyes Sparidae. Northeast Gulf Sci. 12, 99–110.
Cody, R.P., Bortone, S.A., 1997. Masculinization of mosquitofish as an indicator of exposure to kraft mill effluent. Bull. Environ. Contam. Toxicol. 58, 429–436.
Coetzee, P.S., 1983. Seasonal histological and macroscopic changes in the gonads of *Cheimerius nufar* (Ehrenberg, 1820) (Sparidae: Pisces). S. Afr. J. Zool. 18, 76–88.
Cokendolpher, J.C., 1980. Hybridization experiments with the genus *Cyprinodon* (Teleostei: Cyprinodontidae). Copeia 1980, 173–176.
Cole, K.S., 1990. Patterns of gonad structure in hermaphroditic gobies Teleostei Gobiidae. Environ. Biol. Fishes 28, 125–142.
Cole, K.S., Noakes, D.L.G., 1997. Gonadal development and sexual allocation in mangrove killifish, *Rivulus marmoratus* (Pisces: Atherinomorpha). Copeia 1997, 596–600.
Cole, K.S., Robertson, D.R., 1988. Protogyny in the Caribbean reef goby, *Coryphopterus personatus*: gonad ontogeny and social influences on sex-change. Bull. Mar. Sci. 42, 317–333.
Cole, K.S., Shapiro, D.Y., 1990. Gonad structure and hermaphroditism in the gobiid genus *Coryphopterus* Teleostei Gobiidae. Copeia 1990, 996–1003.
Cole, K.S., Shapiro, D.Y., 1992. Gonadal structure and population characteristics of the protogynous goby *Coryphopterus glaucofraenum*. Mar. Biol. 113, 1–9.
Cole, K.S., Shapiro, D.Y., 1995. Social facilitation and sensory mediation of adult sex change in a cryptic, benthic marine goby. J. Exp. Mar. Biol. Ecol. 186, 65–75.
Cole, K.S., Robertson, D.R., Cedeno, A.A., 1994. Does gonad structure reflect sexual pattern in all gobiid fishes? Environ. Biol. Fishes 41, 301–309.
Coleman, F.C., Koenig, C.C., Collins, L.A., 1996. Reproductive styles of shallow-water groupers (Pisces: Serranidae) in the eastern Gulf of Mexico and the consequences of fishing spawning aggregations. Environ. Biol. Fishes 47, 129–141.
Colihueque, N., Iturra, P., Estay, F., Diaz, N.F., 2001. Diploid chromosome number variations and sex chromosome polymorphism in five cultured strains of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 198, 63–77.
Colin, P.L., 1992. Reproduction of the nassau grouper *Epinephelus striatus* Pisces Serranidae and its relationship to environmental conditions. Environ. Biol. Fishes 34, 357–377.
Collares-Pereira, M.J., 1985. The *Rutilus alburnoides* (Steindachner, 1866) complex (Pisces, Cyprinidae): first data on the karyology of a well-established diploid-triploid group. Arq. Mus. Bocage, Ser. A 3, 1–22.
Collares-Pereira, M.J., Madeira, J.M., Rab, P., 1995. Spontaneous triploidy in the stone loach *Noemacheilus barbatulus* (Balitoridae). Copeia 1995, 483–484.
Collares-Pereira, M.J., Prospero, M.I., Bileu, R.I., Rodrigues, E.M., 1998. *Leuciscus* (Pisces, Cyprinidae) karyotypes: transect of Portuguese populations. Genet. Mol. Biol. 21, 63–69.
Colombo, G., Grandi, G., 1989. Observations on the effects of sex steroids on gonadal differentiation in *Anguilla anguilla* L. Acta Embryol. Morphol. Exp. 10, 67–74.
Colombo, G., Grandi, G., 1990. Gonad sex differentiation of *Anguilla anguilla* by sex steroids. Int. Rev. Gesamten Hydrobiol. 75, 763–773.
Colombo, G., Grandi, G., 1995. Sex differentiation in the European eel: histological analysis of the effects of sex steroids on the gonad. J. Fish Biol. 47, 394–413.
Colombo, G., Grandi, G., 1996. Histological study of the development and sex differentiation of the gonad in the European eel. J. Fish Biol. 48, 493–512.
Condeça, J.A.B., Canario, A.V.M., 1995. Steroidogenesis during estrogen-induced sex inversion in the seabream, *Sparus aurata*. Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish, Fish Symposium 95, Austin, TX (USA), pp. 306.
Conover, D.O., 1984. Adaptive significance of temperature-dependent sex determination in a fish. Am. Nat. 123, 297–313.
Conover, D.O., Demond, S.B., 1991. Absence of temperature-dependent sex determination in northern populations of two cyprinodontid fishes. Can. J. Zool. 69, 530–533.

Conover, D.O., Fleisher, M.H., 1986. Temperature-sensitive period of sex determination in the Atlantic silverside, *Menidia menidia*. Can. J. Fish. Aquat. Sci. 43, 514–520.

Conover, D.O., Heins, S.W., 1987a. The environmental and genetic components of sex ratio in *Menidia menidia* (Pisces: Atherinidae). Copeia 1987, 732–743.

Conover, D.O., Heins, S.W., 1987b. Adaptive variation in environmental and genetic sex determination in a fish. Nature 326, 496–498.

Conover, D.O., Kynard, B.E., 1981. Environmental sex determination: interaction of temperature and genotype in a fish. Science 213, 577–579.

Conover, D.O., Van Voorhees, D.A., 1990. Evolution of a balanced sex ratio by frequency-dependent selection in a fish. Science 250, 1556–1558.

Conover, D.O., Van Voorhees, D.A., Ehtisham, A., 1992. Sex ratio selection and the evolution of environmental sex determination in laboratory populations of *Menidia menidia*. Evolution 46, 1722–1730.

Corley Smith, G.E., Lim, C.J., Brandhorst, B.P., 1996. Production of androgenetic zebrafish (*Danio rerio*). Genetics 142, 1265–1276.

Coughlan, T., Schartl, M., Hornung, U., Hope, I., Stewart, A., 1999. PCR-based sex test for *Xiphophorus maculatus*. J. Fish Biol. 54, 218–222.

Cousin-Gerber, M., G, B., C, B., B, C., 1989. Effect of methyltestosterone on sex differentiation and gonad morphogenesis in rainbow trout *Oncorhynchus mykiss*. Aquat. Living Resour. 2, 230–255.

Cowen, R.K., 1990. Sex change and life history patterns of the labrid *Semicossyphus pulcher* across an environmental gradient. Copeia 1990, 787–795.

Craig, J.K., Foote, C.J., Wood, C.C., 1996. Evidence for temperature-dependent sex determination in sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. 53, 141–147.

Crews, D., 1996. Temperature-dependent sex determination: the interplay of steroid hormones and temperature. Zool. Sci. 13, 1–13.

Crews, D., Bergeron, J.M., 1994. Role of reductase and aromatase in sex determination in the red-eared slider (*Trachemys scripta*), a turtle with temperature-dependent sex determination. J. Endocrinol. 143, 279–289.

Dabrowski, K., Rinchard, J., Lin, F., Garcia-Abiado, M.A., Schmidt, D., 2000. Induction of gynogenesis in muskellunge with irradiated sperm of yellow perch proves diploid muskellunge male homogamety. J. Exp. Zool. 287, 96–105.

Da Cruz-Huefling, M., Da Cruz-Landim, C., 1984. Ultrastructural and histochemical studies on the Leydig and Sertoli cell homologues in the testis of *Triportheus elongatus* (Sardinhao) and *Mylossoma aureum* (Pacu). Cytobios 41, 161–174.

Das, R.K., Kar, R.N., 1977. Somatic chromosome analysis of a siluroid fish, *Rita chrysea* Day. Caryologia 30, 247–253.

Das, B., Srivastava, M.D.L., 1973. The meiotic chromosomes of certain teleosts. Proc. Natl. Acad. Sci., India 43, 17–25.

Das, P., Mukhopadhyay, M.K., Das, K.M., Pandit, P.K., 1987. Gonadal sex manipulation of *Oreochromis mossambicus* (Peters). Sel. Hybrid. Genet. Eng. Aquacult. 18, 18–19.

Das, S.K., Shetty, H.P.C., Nandeesha, M.C., 1990. Production of female-free common carp, *Cyprinus carpio* var. communis (L.) through dietary administration of the androgen mibolerone. Asian Fish. Sci. 3, 197–203.

Das, S.K., Shetty, H.P.C., Nandeesha, M.C., DePauw, N., Joyce, J., 1991. Optimization of mibolerone treatment through dietary administration for production of female-free population of common carp. Aquacult. Environ. 14, 86.

Das Neves Falcao, J., Bertollo, L.A., 1985. Chromosome characterization in *Acestrorhynchinae* and *Cynopotaminae* (Pisces, Characidae). J. Fish Biol. 27, 603–610.

Dass, C.C., 1983. Cited in Rishi, K.K. Current status of fish cytogenetics. In: Das and Jhingram (Eds.), Fish Genetics in India, Proc. 70th Indian Sci. Cong. vol. 2. Today and Tomorrow's Printers, New Delhi, India, pp. 1.

Davis, K.B., Simco, B.A., Goudie, C.A., Parker, N.C., Cauldwell, W., Snellgrove, R., 1990. Hormonal sex manipulation and evidence for female homogamety in channel catfish. Gen. Comp. Endocrinol. 78, 218–223.

Davis, K.B., Goudie, C.A., Simco, B.A., Tiersch, T.R., Carmichael, G.J., 1992. Influence of dihydrotestosterone on sex determination in channel catfish and blue catfish period of developmental sensitivity. Gen. Comp. Endocrinol. 86, 147–151.

Davis, K.B., Morrison, J., Galvez, J.I., 2000. Reproductive characteristics of adult channel catfish treated with trenbolone acetate during the phenocritical period of sex differentiation. Aquaculture 189, 351–360.

Dawley, R., Yeakel, A., 1991. Low clonal diversity among unisexual hybrids of the killifishes *Fundulus heteroclitus* and *F. diaphanus*. Am. Zool. 31, 49A.

Dawley, R.M., Graham, J.H., Schultz, R.J., 1985. Triploid progeny of pumpkinseed × green sunfish hybrids. J. Hered. 76, 251–257.

Dawson, C.E., Heal, E., 1977. A bibliography of anomalies of fishes. Gulf Res. Rep. 5, 35–41.

D'Cotta, H., Fostier, A., Guiguen, Y., Govoroun, M., Baroiller, J.-F., 2001. Aromatase plays a key role during normal and temperature-induced sex differentiation of tilapia *Oreochromis niloticus*. Mol. Reprod. Dev. 59, 265–276.

de Almeida Toledo, L.F., Foresti, H., de Almeida Toledo Filho, S., 1984. Complex sex chromosome system in *Eigenmannia* sp. (Pisces, Gymnotiformes). Genetica 64, 165–169.

Debas, L., Fostier, A., Fuchs, J., Weppe, M., Nedelec, G., Benett, A., Cauty, C., Jalabert, B., 1989. The sexuality of cultured hermaphroditic fish species: analysis of morphological and endocrinological features in a protogynous hermaphrodite, *Epinephelus microdon*, as a basis for further research to control reproduction in the grouper. Advances In Tropical Aquaculture: Workshop Held In Tahiti, French Polynesia, February, Actes Colloq. IFREMER, Tahiti, French Polynesia, p. 9.

de Jesus, C., Moreira-Filho, O., 2000. Karyotypes of three species of *Parodon* (Teleostei: Parodontidae). Ichthyol. Explor. Freshwaters (Munchen) 11, 75–80.

de Jesus, C.M., Bertollo, L.A., Moreira-Filho, O., 1999. Comparative cytogenetics in *Apareiodon affinis* (Pisces, Characiformes) and considerations regarding diversification of the group. Genetica 105, 63–67.

Degani, G., Kushnirov, D., 1992. Effects of 17-beta estradiol and grouping on sex determination of European eels. Prog. Fish-Cult. 54, 88–91.

del Carmen Maldonado, M.M., Uribe-Alcocer, M., Arreguin-Espinosa, J., Castro-Perez, A., 1985. Karyotypical studies on *Dormitator maculatus* bloch and gobiomorus dormitor lacepede (Gobiidae: Perciformes). Cytologia 50, 663–669.

Delgado Bermejo, J.V., Moreno Millan, M., 1988. Chromosomes of *Valencia lozanoi* (Pisces, Cyprinodontidae). Cytobios 54, 218–219.

Demska-Zakes, K., Zakes, Z., 1997. Effect of 17 alpha-methyltestosterone on gonadal differentiation in pikeperch, *Stizostedion lucioperca* L. Aquacult. Res. 28, 59–63.

Demska-Zakes, K., Luczynski, M.J., Dabrowski, K., Luczynski, M., Krol, J., 2000. Masculinization of northern pike fry using the steroid 11 beta-hydroxyandrostenedione. North Am. J. Aquacult. 62, 294–299.

Denton, T.E., Howell, W.M., McCollum, C.J., Marks, E.B., Allison, J.J., 1985. Masculinization of female mosquitofish by exposure to plant sterols and *Mycobacterium smegmatis*. Bull. Environ. Contam. Toxicol. 35, 627–632.

Dergam, J.A., Bertollo, L.A.C., 1990. Karyotypic diversification in *Hoplias malabaricus* osteichthyes erythrinidae of the Sao Francisco and Alto Parana Basins Brazil. Rev. Bras. Genet. 13, 755–766.

Desprez, D., Melard, C., 1998a. Influence of sexual genotype on reproduction traits of females (genotype WZ) and pseudofemales (genotype ZZ) in the tilapia *Oreochromis aureus*. Aquat. Living Resour. 11, 145–153.

Desprez, D., Melard, C., 1998b. Effect of ambient water temperature on sex determinism in the blue tilapia *Oreochromis aureus*. Aquaculture 162, 1–2.

Desprez, D., Melard, C., Philippart, J.C., 1995. Production of a high percentage of male offspring with 17 alpha-ethynylestradiol sex-reversed *Oreochromis aureus*: 2. Comparative reproductive biology of females and F2 pseudofemales and large-scale production of male progeny. Aquaculture 130, 35–41.

Devlin, R.H., 1993. Sequence of sockeye salmon Type 1 and 2 growth hormone genes and the relationship of rainbow trout with Atlantic and Pacific salmon. Can. J. Fish. Aquat. Sci. 50, 1738–1748.

Devlin, R.H., Holm, D.G., Grigliatti, T.A., 1988. The influence of whole-arm trisomy on gene expression in *Drosophila*. Genetics 118, 87–101.

Devlin, R.H., Biagi, C.A., Smailus, D.E., 2001. Genetic mapping of Y-chromosomal DNA markers in Pacific salmon. Genetica 111, 43–58.

Devlin, R.H., NcNeil, B.K., Groves, T.D.D., Donaldson, E.M., 1991. Isolation of a Y-chromosomal DNA probe capable of determining genetic sex in chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 48, 1606–1612.

Devlin, R.H., McNeil, B.K., Solar, I., Donaldson, E.M., 1994. A rapid PCR-based test for Y-chromosomal DNA allows simple production of all-female strains of chinook salmon. Aquaculture 128, 211–220.

Devlin, R.H., Stone, G.W., Smailus, D.E., 1998. Extensive direct-tandem organization of a long repeat DNA sequence on the Y chromosome of chinook salmon (*Oncorhynchus tshawytscha*). J. Mol. Evol. 46, 277–287.

Ding, J., Shan, S.X., Ge, W., Jiang, Y.G., 1992. Study on the mode of primary control in the egg of gynogenetic crucian carp for inhibiting the development of two types of sperm nuclei. Sci. China, Ser. B 35, 802–810.

Ding, J.L., Ng, W.K., Lim, E.H., Lam, T.J., 1993. In situ hybridization shows the tissue distribution of vitellogenin gene expression in *Oreochromis aureus*. Cytobios 73, 294–295.

Dipper, F.A., Pullin, R.S.V., 1979. Gonochorism and sex-inversion in British Labridae (Pisces). J. Zool. 187, 97–112.

Disney, J.E., Johnson, K.R., Thorgaard, G.H., 1987. Intergeneric gene transfer of six isozyme loci in rainbow trout by sperm chromosome fragmentation and gynogenesis. J. Exp. Zool. 244, 151–158.

Dlugosz, M., Demska-Zakes, K., 1990. Sex differentiation in European whitefish (*Coregonus lavaretus* L.). Biol. Manage. Coregonid Fishes 39, 3–4.

Docker, M.F., Beamish, F.W.B., 1994. Age, growth, and sex ratio among populations of least brook lamprey, *Lampetra aepyptera*, larvae: an argument for environmental sex determination. Environ. Biol. Fishes 41, 191–205.

Donahue, W.H.A., 1974. A karyotypic study of three species of rajiformes (Chondrichthyes, Pisces). Can. J. Genet. Cytol. 16, 203–211.

Donaldson, E.M., 1996. Manipulation of reproduction in farmed fish. Anim. Reprod. Sci. 42, 381–392.

Donaldson, E.M., Devlin, R.H., 1996. Uses of biotechnology to enhance production. In: Pennell, W., Barton, B. (Eds.), Principles of Salmonid Culture. Elsevier, pp. 969–1020.

Donaldson, E., Hunter, G., 1982. Sex control in fish with particular reference to salmonids. Can. J. Fish. Aquat. Sci. 39, 99–110.

Donaldson, E.M., Hunter, G.A., Dye, H.M., 1981. Induced ovulation in coho salmon (*Oncorhynchus kisutch*): 3. Preliminary study on the use of the antiestrogen tamoxifen. Aquaculture 26, 143–154.

Dong, S., Taniguchi, N., Tsuji, S., 1996. Identification of clones of ginbuna *Carassius langsdorfi* by DNA fingerprinting and isozyme pattern. Nippon Suisan Gakkaishi 62, 747–753.

Donohoe, R.M., Curtis, L.R., 1996. Estrogenic activity of chlordecone, *o,p'* -DDT and *o,p'* -DDE in juvenile rainbow trout: induction of vitellogenesis and interaction with hepatic estrogen binding sites. Aquat. Toxicol. 36, 31–52.

Dreze, V., Monod, G., Cravedi, J.-P., Biagianti-Risbourg, S., Le, G.F., 2000. Effects of 4-nonylphenol on sex differentiation and puberty in mosquitofish (*Gambusia holbrooki*). Ecotoxicology 9, 93–103.

Drori, S., Ofir, M., Levavi-Sivan, B., Yaron, Z., 1994. Spawning induction in common carp (*Cyprinus carpio*) using pituitary extract or GnRH superactive analogue combined with metoclopramide: analysis of hormone profile, progress of oocyte maturation and dependence on temperature. Aquaculture 119, 393–407.

Drysdale, D.T., Bortone, S.A., 1989. Laboratory induction of intersexuality in the mosquitofish, *Gambusia affinis*, using paper mill effluent. Bull. Environ. Contam. Toxicol. 43, 611–617.

Du, S.J., Devlin, R.H., Hew, C.L., 1993. Genomic structure of growth hormone genes in chinook salmon (*Oncorhynchus tshawytscha*): presence of two functional genes, GH-I and GH-II, and a male-specific pseudogene, GH-psi. DNA Cell Biol. 12, 739–751.

Duan, C., Duguay, S., Plisetskaya, E., 1993. Insulin-like growth factor I (IGF-I) mRNA expression in coho salmon, *Oncorhynchus kisutch*: tissue distribution and effects of growth hormone/prolactin family proteins. Fish Physiol. Biochem. 11, 371–379.

Duchac, B.J., Buehler, E.M., 1983. Expression of H–Y antigen in the sex-change fish *Coris julis*. Experientia 39, 767–769.

Duchac, B., Huber, F., Mueller, H., Senn, D., 1982. Mating behaviour and cytogenetical aspects of sex-inversion in the fish *Coris julis* L. (Labridae, Teleostei). Experientia 38, 809–810.

Duda, P., Linhart, O., 1992. The effect of androgens on the early development of common carp (*Cyprinus carpio*) and tench (*Tinca tinca*). Proceedings of the Scientific Conference Fish Reproduction '92, Res. Inst. of Fish Culture and Hydrobiol., Vodnany, Czechoslovakia, pp. 87–88.

Dulcic, J., Kraljevic, M., 1996. Growth of the black sea bream *Spondyliosoma cantharus* (L.) in the eastern middle Adriatic. Arch. Fish. Mar. Res. 44, 279–293.

Duran Gonzalez, A.L., Laguarda Figueras, A., 1992. Cytogenetic characterization of the sailfish *Tetrapturus albidus* Poey, 1860 (Pisces: Istiophoridae) from the Mexican Caribbean Sea. An. Inst. Cienc. Mar. Limnol. 19, 143–150.

Duran Gonzalez, A., Carcia Ruelas, C.E., Laguarda Figueras, A., 1990. The karyotype and "G" bands of *Haemulon aurolineatum* Cuvier, 1829 (Pisces: Haemulidae). An. Inst. Cienc. Mar. Limnol. 17, 299–307.

Ebeling, A.W., Chen, T.R., 1970. Heterogamety in teleostean fishes. Trans. Am. Fish. Soc. 99, 131–138.

Ebeling, A.W., Atkin, N.B., Setzer, P.Y., 1971. Genome sizes of teleostean fishes: increases in some deep-sea species. Am. Nat. 105, 549–561.

Ebisawa, A., Kanashiro, K., Kyan, T., Motonaga, F., 1995. Aspects of reproduction and sexuality in the black-spot Tuskfish, *Choerodon schoenleinii*. Jpn. J. Ichthyol. 42, 121–130.

Ebrahimi, M., Kime, D.E., 1998. Extragonadal steroidogenesis in teleost fish. Ann. N. Y. Acad. Sci. 839, 581–583.

Echelle, A.A., Mosier, D.T., 1982. *Menidia clarkhubbsi*, n. sp. (Pisces: Atherinidea), an all-female species. Copeia 1982, 533–540.

Echelle, A., Echelle, A., DeBault, L., Durham, D., 1988. Ploidy levels in silverside fishes (Atherinidae, *Menidia*) on the Texas coast: flow cytometric analysis of the occurance of allotriploidy. J. Fish Biol. 32, 835–844.

Eck, G.W., Allen, J.D., 1993. The effects of temperature on sex determination in the bloater *Coregonus hoyi*: a hypothesis test. Proceedings of the Fifth International Symposium Adv. Limnol. vol. 46, Stuttgart: E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart (FRG), pp. 173–179.

Eckstein, B., Abraham, M., Zohar, Y., 1978. Production of steroid hormones by male and female gonads of *Sparus aurata* (Teleostei, Sparidae). Comp. Biochem. Physiol. 60B, 93–97.

Edmunds, J., 1999. Effects of xenoestrogens and cytochrome *P*450 aromatase inhibitors on sexual differentiation in medaka (*Oryzias latipes*). Diss. Abstr. Int., B 60, 1387.

Elder Jr., J.F., Turner, B.J., Thomerson, J.E., Taphorn, D.C. 1991. Chromosomal divergence and heterogameity in two annual killifishes of the genus *Pterolebias*. Genome 34, 674–676.

Elizur, A., Meiri, I., Rosenfeld, H., Zmora, N., Knibb, W.R., Zohar, Y., 1995. Seabream gonadotropins: sexual dimorphism in gene expression. Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish, Fish Symposium 95, Austin, TX (USA), pp. 13–15.

Elofsson, U., Winberg, S., Francis, R.C., 1997. Number of preoptic GnRH-immunoreactive cells correlates with sexual phase in a protandrously hermaphroditic fish, the dusky anemonefish (*Amphiprion melanopus*). J. Comp. Physiol. A 181, 484–492.

Elskus, A.A., Pruell, R., Stegeman, J.J., 1992. Endogenously mediated, pretranslational suppression of cytochrome *P*4501A in PCB-contaminated flounder. Respir. Mar. Org. Pollut., 1–4, Part 34.

Epler, P., Galas, J., Stoklosowa, S., 1997. Steroidogenic activity of carp ovarian follicular and interstitial cells at the pre-spawning and resting time: a tissue culture approach. Comp. Biochem. Physiol. C 116C, 167–170.

Erickson, D.L., Grossman, G.D., 1986. Reproductive demography of tilefish from the South Atlantic Bight with a test for the presence of protogynous hermaphroditism. Trans. Am. Fish. Soc. 115, 279–285.

Estay, F., Neira, R., Diaz, N.F., Valladares, L., Torres, A., 1998. Gametogenesis and sex steroid profiles in cultured coho salmon (*Oncorhynchus kisutch*, Walbaum). J. Exp. Zool. 280, 429–438.

Ewulonu, U.K., 1987. Molecular analysis of heterogamety and sex determining mechanisms in teleost fishes. Diss. Abstr. Int., B 48, 1–97.

Ewulonu, U.K., Haas, R., Turner, B.J., 1985. A multiple sex chromosome system in the annual killifish, *Nothobranchius guentheri*. Copeia 1985, 503–508.

Farr, J.A., 1981. Biased sex ratios in laboratory strains of guppies, *Poecilia reticulata*. Heredity 47, 237–248.

Fedorov, K.E., Zubova, S.E., Semenov, V.V., Burlakov, A.B., 1990. Secretory cells in the gonads of the young sterlet *Acipenser ruthenus* during sex differentiation. Vopr. Ikhtiol. 30, 65–75.

Feist, G., Schreck, C.B., 1996. Brain–pituitary–gonadal axis during early development and sexual differentiation in the rainbow trout, *Oncorhynchus mykiss*. Gen. Comp. Endocrinol. 102, 394–409.

Feist, G., Schreck, C.B., Fitzpatrick, M.S., Redding, J.M., 1990. Sex steroid profiles of coho salmon (*Oncorhynchus kisutch*) during early development and sexual differentiation. Gen. Comp. Endocrinol. 80, 299–313.

Feist, G., Yeoh, C.-G., Fitzpatrick, M.S., Schreck, C.B., 1995. The production of functional sex-reversed male rainbow trout with 17 alpha-methyltestosterone and 11 beta-hydroxyandrostenedione. Aquaculture 131, 1–2.

Feldberg, E., Bertollo, L.A.C., de Almeida Toledo, L.F., Foresti, F., Filho, O.M., Dos Santos, A.F., 1987. Biological aspects of Amazonian fishes: IX. Cytogenetic studies in two species of the genus *Semaprochilodus* (Pisces, Prochilodontidae). Genome 29, 1–4.

Felip, A., Zanuy, S., Carrillo, M., Piferrer, F., 1999. Growth and gonadal development in triploid sea bass (*Dicentrarchus labrax* L.) during the first two years of age. Aquaculture 173, 387–397.

Fenocchio, A.S., Bertollo, L.A.C., 1992. Karyotype C-bands and nors of the neotropical siluriform fish *Ageneiosus brevifilis* and *Ageneiosus atronases* Ageneiosidae. Cytobios 72, 19–22.

Fernando, A.A., Phang, V.P.E., 1989. Inheritance of the tuxedo and blond tuxedo color pattern phenotypes of the guppy, *Poecilia reticulata*. Proceedings of The Second Asian Fisheries Forum, Tokyo, Japan, The Second Asian Fisheries Forum, Tokyo, Japan, pp. 487–490.

Fernando, A.A., Phang, V.P.E., 1990. Colour pattern inheritance in three domesticated varieties of guppy, *Poecilia reticulata*. Genet. Aquacult. III 85, 320.

Ferreira, B.P., 1993. Reproduction of the inshore coral trout *Plectropomus maculatus* Perciformes Serranidae from the Central Great Barrier Reef Australia. J. Fish Biol. 42, 831–844.

Ferreira, B.P., Russ, G.R., 1995. Population structure of the leopard coralgrouper, *Plectropomus leopardus*, on fished and unfished reefs off Townsville, Central Great Barrier Reef, Australia. U.S. Natl. Mar. Fish. Serv. Fish. Bull.-NOAA 93, 629–642.

Ferreiro, C., Medrano, J.F., Gall, G.A.E., 1989. Genome analysis of rainbow trout and sturgeon with restriction enzymes and hybridization with a *Zfy* gene derived probe to identify sex. Aquaculture 81, 245–252.

Fine, M.L., Keefer, D.A., Russel-Mergenthal, H., 1990. Autoradiographic localization of estrogen-concentrating cells in the brain and pituitary of the oyster toadfish. Brain Res. 536, 207–219.

Fineman, R., Hamilton, J., Chase, G., 1975. Reproductive performance of male and female phenotypes in three sex chromosomal genotypes (XX, XY, YY) in the killifish, *Oryzias latipes*. J. Exp. Zool. 192, 349–354.

Fischer, E.A., Petersen, C.W., 1987. The evolution of sexual patterns in the seabasses. Bioscience 37, 482–489.

Fishelson, L., 1970. Protogynous sex reversal in the fish *Anthias squamipinnis* (Teleostei, Anthiidae) regulated by the presence or absence of a male fish. Nature 227, 90–91.

Fishelson, L., 1990. *Rhinomuraena* spp. (Pisces: Muraenidae): the first vertebrate genus with post-anally situated urogenital organs. Mar. Biol. 105, 253–257.

Fishelson, L., 1992. Comparative gonad morphology and sexuality of the Muraenidae Pisces Teleostei. Copeia 1992, 197–209.

Fisher, R.A., 1930. The Genetical Theory of Natural Selection. Dover, New York.

Fitzpatrick, M.S., 1985. Endocrine mediation of reproduction in coho salmon (*Oncorhynchus kisutch*). Publ. Oreg. State Univ. Sea Grant Coll. Program, 4.

Fitzpatrick, M.S., Redding, J.M., Ratti, F.D., Schreck, C.B., 1987. Plasma testosterone concentration predicts the ovulatory response of coho salmon (*Oncorhynchus kisutch*) to gonadotropin-releasing hormone analog. Can. J. Fish. Aquat. Sci. 44, 1351–1357.

Fitzpatrick, M.S., Pereira, C.B., Schreck, C.B., 1993. In vitro steroid secretion during early development of mono-sex rainbow trout: sex differences, onset of pituitary control, and effects of dietary steroid treatment. Gen. Comp. Endocrinol. 91, 199–215.

Fitzpatrick, M.S., Gale, M.L., Schreck, C.B., 1994. Binding characteristics of an androgen receptor in the ovaries of coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. 95, 399–408.

Fitzpatrick, M.S., Gale, W.L., Slater, C.H., Schreck, C.B., 1995. Gonadal androgen receptors in fishes. Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish, Fish Symposium 95, Austin, TX (USA), p. 308.

Flores, J.A., Burns, J.R., 1993. Ultrastructural study of embryonic and early adult germ cells and their support cells in both sexes of *Xiphophorus* Teleostei Poecillidae. Cell Tissue Res. 271, 263–270.

Flouriot, G., Pakdel, F., Ducouret, B., Valotaire, Y., 1995. Influence of xenobiotics on rainbow trout liver estrogen receptor and vitellogenin gene expression. J. Mol. Endocrinol. 15, 143–151.

Flouriot, G., Pakdel, F., Valotaire, Y., 1996. Transcriptional and post-transcriptional regulation of rainbow trout estrogen receptor and vitellogenin gene expression. Mol. Cell. Endocrinol. 124, 173–183.

Foerster, W., Anders, F., 1977. Chromosome complements from different subspecies and species of *Xiphophorus*. Zool. Anz. 198, 167–177.

Forbes, S.H., Knudsen, K.L., North, T.W., Allendorf, F.W., 1994. One of two growth hormone genes in coho salmon is sex-linked. Proc. Natl. Acad. Sci. U. S. A. 91, 1628–1631.

Foresti, F., Almeida Toledo, L.F., Pathak, S., 1983. Silver-stained NOR and synaptonemal complex analysis during male meiosis of *Tilapia rendalli*. J. Hered. 74, 127–128.

Foucher, J.L., Le Gac, F., 1989. Evidence for an androgen binding protein in the testis of a teleost fish (*Salmo gairdneri* R.): a potential marker of Sertoli cell function. J. Steroid Biochem. 32, 545–552.

Foucher, J.L., de Niu, P., Mourot, B., Vaillant, C., Le Gac, F., 1991. In-vivo and in-vitro studies on sex steroid binding protein sbp regulation in rainbow trout *Oncorhynchus mykiss* influence of sex steroid hormones and of factors linked to growth and metabolism. J. Steroid Biochem. 39, 975–986.

Foucher, J.L., Le Bail, P.Y., Le Gac, F., 1992. Influence of hypophysectomy castration fasting and spermiation on Sbp concentration in male rainbow trout *Oncorhynchus mykiss*. Gen. Comp. Endocrinol. 85, 101–110.

Foyle, T.P., 1993. A histological description of gonadal development and sex differentiation in the coho salmon (*Oncorhynchus kisutch*) for both untreated and oestradiol immersed fry. J. Fish Biol. 42, 699–712.

Francis, R.C., Barlow, G.W., 1993. Social control of primary sex differentiation in the Midas cichlid. Proc. Natl. Acad. Sci. U. S. A. 90, 10673–10675.

Francis, R.C., Soma, K., Fernald, R.D., 1993. Social regulation of the brain–pituitary–gonadal axis. Proc. Natl. Acad. Sci. U. S. A. 90, 7791–7798.

Frantzen, M., Johnsen, H.K., Mayer, I., 1997. Gonadal development and sex steroids in a female Arctic charr broodstock. J. Fish Biol. 51, 697–709.

Fraser, D., 1997. A hermaphroditic Arctic charr from Loch Rannoch, Scotland. J. Fish Biol. 50, 1358–1359.

Fricke, H., 1979. Mating system, resource defence and sex change in the anemonefish *Amphiprion akallopisos*. Z. Tierpsychol. 50, 313–326.

Fricke, H.W., 1983. Social control of sex: field experiments with the anemonefish *Amphiprion bicinctus*. Z. Tierpsychol. 61, 71–77.

Fricke, H., Fricke, S., 1977. Monogamy and sex change by aggressive dominance in coral reef fish. Nature 266, 830–832.

Frolov, S.B., 1989. Differentiation of sex chromosomes in the salmonidae I, karyotype and sex chromosomes in *Parasalmo mykiss*. Tsitologiya 31, 1391–1394.

Frolov, S.V., 1990. Differentiation of sex chromosomes in salmonidae: III. Multiple sex chromosomes in *Coregonus sardinella*. Tsitologiya 32, 659–663.

Fujio, Y., MacAranas, J.M., 1989. Detection of a null allele for MDH isozymes in the guppy (*Poecilia reticulata*), with special reference to sex-linked inheritance. Jpn. J. Genet. 64, 347–354.

Fujio, Y., Nakajima, M., Nagahama, Y., 1990. Detection of a low temperature-resistant gene in the guppy (*Poecilia reticulata*), with reference to sex-linked inheritance. Jpn. J. Genet. 65, 201–207.

Fujioka, Y., 1993. Sex reversal in honmoroko, *Gnathpogon caerulescens* by immersion in 17-methyltestosterone and an attempt to produce all-female progeny. Suisan Zoshoku 41, 409–416.

Fujioka, Y., 1998. Survival, growth and sex ratios of gynogenetic diploid honmoroko. J. Fish Biol. 52, 430–442.

Fujioka, Y., 2001. Thermolabile sex determination in honmoroko. J. Fish Biol. 59, 851–861.

Fujiwara, Y., Hirabayashi, T., Miyazaki, J.I., 1992. Search for factors related to sex differentiation in fish. Zool. Sci. 9, 1173.

Fukada, S., Tanaka, M., Iwaya, M., Nakajima, M., Nagahama, Y., 1995. The *Sox* gene family and its expression during embryogenesis in the teleost fish, medaka (*Oryzias latipes*). Dev., Growth Differ. 37, 379–385.

Fukada, S., Tanaka, M., Matsuyama, M., Kobayashi, D., Nagahama, Y., 1996. Isolation, characterization, and expression of cDNAs encoding the medaka (*Oryzias latipes*) ovarian follicle cytochrome *P*-450 aromatase. Mol. Reprod. Dev. 45, 285–290.

Fukui, Y., Gushima, K., Kakuda, S., Hashimoto, H., 1991. Growth-related changes in color and sex in *Halichoeres poecilopterus*. Jpn. J. Ichthyol. 37, 395–401.

Gajardo, G., Arratia, G., 1981. Analysis of the morphological and chromosomal differentiation of Chilean fish (Pisces: Atherinidae). Arch. Biol. Med. Exp. 14, 57.

Galbreath, P.F., St. Jean, W., Anderson, V., Thorgaard, G.H., 1994. Freshwater performance of all-female diploid and triploid Atlantic salmon. Aquaculture 128, 41–49.

Galbusera, P., Volckaert, F.A.M., Ollevier, F., 2000. Gynogenesis in the African catfish *Clarias gariepinus*

(Burchell, 1822): III. Induction of endomitosis and the presence of residual genetic variation. Aquaculture 185, 25–42.

Gale, W.L., Fitzpatrick, M.S., Schreck, C.B., 1995. Immersion of Nile tilapia (*Oreochromis niloticus*) in 17 alpha-methyltestosterone and mestanolone for the production of all-male populations. Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish, Fish Symposium 95, Austin, TX (USA), p. 117.

Galetti Jr., P.M., Foresti, F., 1986. Evolution of the ZZ/ZW system in *Leporinus* (Pisces, Anostomidae). Cytogenet. Cell Genet. 43, 43–46.

Galetti Jr., P.M., Rasch, E.M., 1993. Chromosome studies in *Poecilia latipunctata* with NOR polymorhism as shown by silver nitrate and chromomycin A sub(3) (Teleostei: Poeciliidae). Ichthyol. Explor. Freshwaters (Munchen) 4, 269–277.

Galetti Jr., P.M., Foresti, F., Bertollo, L.A.C., Moreira Filho, O., 1981. Heteromorphic sex chromosomes in three species of the genus *Leporinus* (Pisces, Anostomidae). Cytogenet. Cell Genet. 29, 138–142.

Galetti Jr., P.M., Lima, N.R.W., Venere, P.C., 1995. A monophyletic ZW sex chromosome system in *Leporinus* (Anostomidae, Characiformes). Cytologia 60, 375–382.

Galvez, J.I., Mazik, P.M., Phelps, R.P., Mulvaney, D.R., 1995. Masculinization of channel catfish *Ictalurus punctatus* by oral administration of trenbolone acetate. J. World Aquacult. Soc. 26, 378–383.

Galvez, J.I., Morrison, J.R., Phelps, R.P., 1996. Efficacy of trenbolone acetate in sex inversion of the blue tilapia *Oreochromis aureus*. J. World Aquacult. Soc. 27, 483–486.

Garcia Cagide, A., Garcia, T., 1996. Reproduction of *Mycteroperca bonaci* and *Mycteroperca venenosa* (Pisces: Serranidae) on the Cuban continental shelf. Rev. Biol. Trop. 44, 771–780.

Garratt, P.A., 1986. Protogynous hermaphroditism in the slinger, *Chrysoblephus puniceus* (Gilchrist and Thompson, 1908) (Teleostei: Sparidae). J. Fish Biol. 28, 297–306.

Garrett, G.P., 1989. Hormonal sex control of largemouth bass. Prog. Fish-Cult. 51, 146–148.

Garratt, P.A., 1993. Spawning of riverbream *Acanthopagrus berda* in Kosi Estuary. S. Afr. J. Zool. 28, 26–31.

Ge, W., Miura, T., Kobayashi, H., Peter, R.E., Nagahama, Y., 1997a. Cloning of cDNA for goldfish activin beta-B subunit and the expression of its mRNA in gonadal and non-gonadal tissues. J. Mol. Endocrinol. 19, 37–45.

Ge, W., Tanaka, M., Yoshikuni, M., Eto, Y., Nagahama, Y., 1997b. Cloning and characterization of goldfish activin type IIB receptor. J. Mol. Endocrinol. 19, 47–57.

George, T., Pandian, T.J., 1995. Production of ZZ females in the female-heterogametic black molly, *Poecilia sphenops*, by endocrine sex reversal and progeny testing. Aquaculture 136, 81–90.

George, T., Pandian, T.J., 1996. Hormonal induction of sex reversal and progeny testing in the zebra cichlid *Cichlasoma nigrofasciatum*. J. Exp. Zool. 275, 374–382.

George, T., Pandian, T.J., 1998. Dietary administration of androgens induces sterility in the female-heterogametic black molly, *Poecilia sphenops* (Cuvier and Valenciennes, 1846). Aquacult. Res. 29, 167–175.

George, T., Pandian, T.J., Kavumpurath, S., 1994. Inviability of YY zygotes of the fighting fish, *Betta splendens*. Isr. J. Aquacult.-Bamidgeh 46, 3–8.

Ghorbel, M., 1996. Common pandora *Pagellus erythrinus* (Pisces, Sparidae). Ecobiology and State of Exploitation in the Gulf of Gabes. Thesis Dissertation, Universite de Sfax, Tunisia, p. 170.

Gillanders, B.M., 1995. Reproductive biology of the protogynous hermaphrodite *Achoerodus viridis* (Labridae) from south-eastern Australia. Mar. Freshwater Res. 46, 999–1008.

Gilling, C.J., Skibinski, D.O.F., Beardmore, J.A., 1996. Sex reversal of tilapia fry by immersion in water containing estrogens. ICLARM Conference Proceedings, Makati City (Philippines). ICLARM, Makati City, Philippines, pp. 314–319.

Gimeno, S., Gerritsen, A., Bowmer, T., Komen, H., 1996. Feminization of male carp. Nature 384, 221–222.

Gimeno, S., Komen, H., Venderbosch, P.W.M., Bowmer, T., 1997. Disruption of sexual differentiation in genetic male common carp (*Cyprinus carpio*) exposed to an alkylphenol during different life stages. Environ. Sci. Technol. 31, 2884–2890.

Gimeno, S., Komen, H., Gerritsen, A.G.M., Bowmer, T., 1998a. Feminisation of young males of the common carp, *Cyprinus carpio*, exposed to 4-*tert*-pentylphenol during sexual differentiation. Aquat. Toxicol. 43, 77–92.

Gimeno, S., Komen, H., Jobling, S., Sumpter, J., Bowmer, T., 1998b. Demasculinisation of sexually mature male

common carp, *Cyprinus carpio*, exposed to 4-*tert*-pentylphenol during spermatogenesis. Aquat. Toxicol. 43, 93–109.

Goddard, K.A., Dawley, R.M., 1990. Clonal inheritance of a diploid nuclear genome by a hybrid freshwater minnow (*Phoxinus eos-neogaeus*, Pisces: Cyprinidae). Evolution 44, 1052–1065.

Goddard, K.A., Megwinoff, O., Wessner, L.L., Giaimo, F., 1998. Confirmation of gynogenesis in *Phoxinus eos-neogaeus* (Pisces: Cyprinidae). J. Hered. 89, 151–157.

Godwin, J., 1994a. Behavioural aspects of protandrous sex change in the anemonefish, *Amphiprion melanopus*, and endocrine correlates. Anim. Behav. 48, 551–567.

Godwin, J., 1994b. Histological aspects of protandrous sex change in the anemonefish *Amphiprion melanopus* (Pomacentridae, Teleostei). J. Zool. 232, 199–213.

Godwin, J., 1995. Phylogenetic and habitat influences on mating system structure in the humbug damselfishes (Dascyllus, Pomacentridae). Bull. Mar. Sci. 57, 637–652.

Godwin, J.R., Thomas, P., 1993. Sex change and steroid profiles in the protandrous anemonefish *Amphiprion melanopus* Pomacentridae Teleostei. Gen. Comp. Endocrinol. 91, 144–157.

Godwin, J., Crews, D., Warner, R.R., 1996. Behavioural sex change in the absence of gonads in a coral reef fish. Proc. R. Soc. London, Ser. B 263, 1683–1688.

Goetz, F.W., 1983. Hormonal Control of Oocyte Final Maturation and Ovulation in Fishes. Academic Press, New York, pp. 117–170.

Goetz, F.W., Garczynski, M., 1997. The ovarian regulation of ovulation in teleost fish. Fish Physiol. Biochem. 17, 33–38.

Goetz, F.W., Donaldson, E.M., Hunter, G.A., Dye, H.M., 1979. Effects of estradiol-17 and 17-methyltestosterone on gonadal differentiation in the coho salmon, *Oncorhynchus kisutch*. Aquaculture 17, 267–278.

Goetz, F.W., Fostier, A.Y., Breton, B., Jalabert, B., 1987. Hormonal changes during meiotic maturation and ovulation in the brook trout (*Salvelinus fontinalis*). Fish Physiol. Biochem. 3, 203–211.

Gold, J.R., Karel, W.J., Strand, M.R., 1980. Chromosome formulae of North American fishes. Prog. Fish-Cult. 42, 10–23.

Gomel'skii, B.I., Cherfas, N.B., 1982. Hormonal reversal of sex in females of a unisexual form of crucian carp. Sov. J. Dev. Biol. 13, 142–148.

Gomelsky, B.I., 1985. Hormonal sex inversion in the carp (*Cyprinus carpio*). Ontogenez 16, 398–405.

Gomelsky, B.I., Emelyanova, O.V., Recoubratsky, A.V., 1992. Application of the scale cover gene (N) to identification of type of gynogenesis and determination of ploidy in common carp. Aquaculture 106, 3–4.

Gomelsky, B., Cherfas, N.B., Peretz, Y., Ben-Dom, N., Hulata, G., 1994. Hormonal sex inversion in the common carp (*Cyprinus carpio* L.). Aquaculture 126, 265–270.

Gomelsky, B., Cherfas, N.B., Gissis, A., Hulata, G., 1999. Hormonal sex inversion in striped bass and white bass X striped bass hybrids. North Am. J. Aquacult. 61, 199–205.

Gomez, C., Vinas, A., Gomez, S., Martinez, P., Sanchez, L., 1993. A comparative karyotipic analysis in both sex individuals of *Anguilla anguilla*. Actas del IV Congreso Nacional de Acuicultura, Pontevedra (Spain). Centro de Investigaciones Marinas, Pontevedra, Spain, pp. 227–232.

Gomez Gaspar, A., 1985. Observations on gonadal development in the hermaphroditic fish *Diplectrum-Formosum* Linnaeus 1758 in Northeastern Venezuela. Bol. Inst. Oceanogr.Venez., Univ. Oriente 24, 129–134.

Goodfellow, P.N., Camerino, G., 2001. DAX-1, an "antitestis" gene. EXS 91, 57–69.

Gorbman, A., 1990. Sex differentiation in the hagfish *Eptatretus stouti*. Gen. Comp. Endocrinol. 77, 309–323.

Gordo, L.S., 1995. Protogynous hermaphroditism in the bogue, *Boops boops* (L.), from the Portuguese coast. Port. Zool. 3, 1–7.

Gordon, M., 1946. Interchanging genetic mechanisms for sex determination. J. Hered. 37, 307–320.

Gordon, M., 1947. Genetics of *Platypoecilus maculatus*: IV. The sex-determining mechanism in two wild populations of of Mexican platyfish. Genetics 32, 8–17.

Gordon, M.R., Owen, T.G., Ternan, T.A., Hildebrand, L.D., 1984. Measurement of a sex-specific protein in skin mucus of premature coho salmon (*Oncorhynchus kisutch*). Aquaculture 43, 1–3.

Gorshkov, S., Gorshkova, G., Knibb, W., Gordin, H., 1999. Sex ratios and growth performance of European sea bass (*Dicentrarchus labrax* L.) reared in mariculture in Eilat (Red Sea). Isr. J. Aquacult. 51, 91–105.

Goryczko, K., Bieniarz, K., Dobosz, S., Grudniewska, J., 1991. The effects of 17-beta estradiol on rainbow trout (*Oncorhynchus mykiss* Walb.). Pol. Arch. Hydrobiol. 38, 303–309.

Goto, R., Mori, T., Kawamata, K., Matsubara, T., Mizuno, S., Adachi, S., Yamauchi, K., 1999. Effects of temperature on gonadal sex determination in barfin flounder *Verasper moseri*. Fish. Sci. 65, 884–887.

Goto, R., Kayaba, T., Adachi, S., Yamauchi, K., 2000a. Effects of temperature on sex determination in marbled sole *Limanda yokohamae*. Fish. Sci. 66, 400–402.

Goto, R., Abe, Y., Masai, K., Yamaha, E., Adachi, S., Yamauchi, K., 2000b. Effects of Environmental Factors on Sex Differentiation in Goldfish *Carassius auratus* University of Bergen, Bergen, Norway.

Goudie, C.A., Redner, B.D., Simco, B.A., Davis, K.B., 1983. Feminization of channel catfish by oral administration of steroid sex hormones. Trans. Am. Fish. Soc. 112, 670–672.

Goudie, C.A., Simco, B.A., Davis, K.B., 1995a. Failure of gynogenetically-derived male channel catfish to produce all-male offspring. Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish, Fish Symposium 95, Austin, TX (USA), p. 118.

Goudie, C.A., Simco, B.A., Davis, K.B., Liu, Q., 1995b. Production of gynogenetic and polyploid catfish by pressure-induced chromosome set manipulation. Aquaculture 133, 3–4.

Govoroun, M., McMeel, O.M., Mecherouki, H., Smith, T.J., Guiguen, Y., 2001. 17beta-Estradiol treatment decreases steroidogenic enzyme messenger ribonucleic acid levels in the rainbow trout testis. Endocrinology 142, 1841–1848.

Gray, M.A., Metcalfe, C.D., 1997. Induction of testis-ova in Japanese medaka (*Oryzias latipes*) exposed to *p*-nonylphenol. Toxicol. Chem. 16, 1082–1085.

Gray, E.S., Woodin, B.R., Stegeman, J.J., 1991. Sex differences in hepatic monooxygenases in winter flounder (*Pseudopleuronectes americanus*) and scup (*Stenotomus chrysops*) and regulation of *P*450 forms by estradiol. J. Exp. Zool. 259, 330–342.

Gray, M.A., Niimi, A.J., Metcalfe, C.D., 1999. Factors affecting the development of testis–ova in medaka, *Oryzias latipes*, exposed to octylphenol. Environ. Toxicol. Chem. 18, 1835–1842.

Greco, T., Payne, A., 1994. Ontogeny of expression of the genes for steroidogenic enzymes *P*450 side-chain cleavage, 3 beta-hydroxysteroid dehydrogenase, *P*450 17 alpha-hydroxylase/C17–20 lyase, and *P*450 aromatase in fetal mouse gonads. Endocrinology 135, 262–268.

Griffiths, R., Orr, K.J., Adam, A., Barber, I., 2000. DNA sex identification in the three-spined stickleback. J. Fish Biol. 57, 1331–1334.

Grober, M.S., Bass, A.H., 1991. Neuronal correlates of sex/role change in labrid fishes: LHRH-like immunoreactivity. Brain Behav. Evol. 38, 302–312.

Grunina, A.S., Nejfakh, A.A., 1991. Induction of androgenetic diploid in Siberian sturgeon *Acipenser baeri* Brandt. Ontogenez 1, 53–56.

Grunina, A.S., Recoubratsky, A.V., Neyfakh, A.A., 1995. Induced diploid androgenesis in sturgeons. Sturgeon Q. 3, 6–7.

Guan, G., Tanaka, M., Todo, T., Young, G., Yoshikuni, M., Nagahama, Y., 1999. Cloning and expression of two carbonyl reductase-like 20beta-hydroxysteroid dehydrogenase cDNAs in ovarian follicles of rainbow trout (*Oncrohynchus mykiss*). Biochem. Biophys. Res. Commun. 255, 123–128.

Guan, G., Kobayashi, T., Nagahama, Y., 2000. Sexually dimorphic expression of two types of DM (Doublesex/Mab-3)-domain genes in a teleost fish, the tilapia (*Oreochromis niloticus*). Biochem. Biophys. Res. Commun. 272, 662–666.

Guerrero, R., 1975. Use of androgens for the production of all-male *Tilapia aurea* (Steinddachner). Trans. Am. Fish. Soc. 2, 342–348.

Guerrero, R.D., 1979. Use of hormonal steroids for artificial sex reversal of Tilapia. Proc. Indian Natl. Sci. Acad., Part B 45, 512–514.

Guerrero III, R.D., Guerrero, L.A. 1993. Effect of oral treatment of mibolerone on sex reversal of *Oreochromis mossambicus*. Asian Fish. Sci. 6, 347–350.

Guiguen, Y., Jalabert, B., Thouard, E., Fostier, A., 1993. Changes in plasma and gonadal steroid hormones in relation to the reproductive cycle and the sex inversion process in the protandrous seabass, *Lates calcarifer*. Gen. Comp. Endocrinol. 92, 327–338.

Guiguen, Y., Cauty, C., Fostier, A., Fuchs, J., Jalabert, B., 1994. Reproductive cycle and sex inversion of the seabass, *Lates calcarifer*, reared in sea cages in French Polynesia: histological and morphometric description. Environ. Biol. Fishes 39, 231–247.

Guiguen, Y., Jalabert, B., Benett, A., Fostier, A., 1995. Gonadal in vitro androstenedione metabolism and changes

in some plasma and gonadal steroid hormones during sex inversion of the protandrous sea bass, *Lates calcarifer*. Gen. Comp. Endocrinol. 100, 106–118.

Guiguen, Y., Baroiller, J.F., Ricordel, M.J., Iseki, K., McMeel, O.M., Martin, S.A.M., Fostier, A., 1999. Involvement of estrogens in the process of sex differentiation in two fish species: the rainbow trout (*Oncorhynchus mykiss*) and a Tilapia (*Oreochromis niloticus*). Mol. Reprod. Dev. 54, 154–162.

Guilherme, L.C., 1992. Progeny-test in *Oreochromis niloticus* (Trewavas, 1982) submitted to sexual inversion. Cienc. Prat. 16, 283–287.

Guraya, S.S., 1994. Gonadal development and production of gametes in fish. Proc. Indian Natl. Sci. Acad., Part B 60, 15–32.

Haaf, T., Scmid, M., 1984. An early stage of ZW/ZZ sex chromosome differentiation in *Poecilia sphenops* var. *melanistica* (Poeciliidae, Cyprinodontoformes). Chromosoma 89, 37–41.

Hackman, E., Reinboth, R., 1974. Delimitation of the critical stage of hormone-influenced sex differentiation in *Pseudocrenilabrus multicolor* (Hilgendorf) (Cichlidae). Gen. Comp. Endocrinol. 22, 42–53.

Haddy, J.A., Pankhurst, N.W., 1998. The dynamics of in vitro 17 beta-estradiol secretion by isolated ovarian follicles of the rainbow trout (*Oncorhynchus mykiss*). Fish Physiol. Biochem. 18, 267–275.

Hamaguchi, S., 1982. A light- and electron-microscopic study on the migration of primordial germ cells in the teleost, *Oryzias latipes*. Cell Tissue Res. 227, 139–151.

Hamaguchi, S., 1992. Sex differentiation of germ cells and their supporting cells in *Oryzias latipes*. Fish Biol. J. 4, 11–17.

Hamaguchi, S., Egami, N., 1980. The male secondary sex characteristics in the gynogenetic female fish, *Poecilia formosa*, induced by the administration of methyltestosterone. Annot. Zool. Jpn. 53, 227–230.

Hamaguchi, S., Sakaizumi, M., 1991. Autosomal genes involved in primary sex determination in the teleost, *Oryzias latipes*. Zool. Sci. 8, 1126.

Hammerman, I.S., Avtalion, R.R., 1979. Sex determination in *Sarotherodon* (Tilapia): Part 2. The sex ratio as a tool for the determination of genotype — a model of autosomal and gonosomal influence. Theor. Appl. Genet. 55, 177–187.

Hansson, T., Gustafsson, J., 1981. Sex differences in the hepatic in vitro metabolism of 4-androstene-3, 17-dione in rainbow trout, *Salmo gairdneri*. Gen. Comp. Endocrinol. 44, 181–188.

Hansson, T., Rafter, J., 1983. in vitro metabolism of estradiol-17 beta by liver microsomes from juvenile rainbow trout, *Salmo gairdneri*. Gen. Comp. Endocrinol. 49, 490–495.

Happe, A., Zohar, Y., 1988. Self-fertilization in the protandrous hermaphrodite *Sparus aurata*: development of the technology. Reproduction in Fish: Basic and Applied Aspects in Endocrinology and Genetics. Colloq. Inst. Natl. Rech. Agron. 44, 177–180.

Haqq, C.M., King, C.Y., Donahoe, P.K., Weiss, M.A., 1993. SRY recognizes conserved DNA sites in sex-specific promoters. Proc. Natl. Acad. Sci. U. S. A. 90, 1097–1101.

Hardisty, M.W., Potter, I.C., Koehn, J.D., 1992. Gonadogenesis and sex differentiation in the southern hemisphere lamprey *Mordacia mordax*. J. Zool. 226, 491–516.

Harrelson, R.A., Rodgers, J.H.J., Lizotte Jr., R.E., Dorn, P.B. 1997. Responses of fish exposed to a C sub(9–11) linear alcohol ethoxylate nonionic surfactant in stream mesocosms. Ecotoxicology 6, 321–333.

Harries, J.E., Sheahan, D.A., Jobling, S., Matthiessen, P., Neall, P., Routledge, E.J., Rycroft, R., Sumpter, J.P., Tylor, T., 1996. A survey of estrogenic activity in United Kingdom inland waters. Environ. Toxicol. Chem. 15, 1993–2002.

Harrington, R.W., 1967. Environmentally controlled induction of primary male gonochorists from eggs of the self-fertilizing hermaphroditic fish, *Rivulus marmoratus* Poey. Biol. Bull. 131, 174–199.

Harrington, R.W., Crossman, R.A., 1976. Effects of temperature and sex genotype on meristic counts of the gonochoristic cyprinodontid fish *Rivulus cylindraceus* Poey. Can. J. Zool. 54, 245–254.

Haskins, C.P., Haskins, E.F., Hewitt, R.E., 1960. Pseudogamy as an evolutionary factor in the Poeciliid fish *Molliensa formosa*. Evolution 14, 473–483.

Hassin, S., de Monbrison, D., Hanin, Y., Elizur, A., Zohar, Y., Popper, D.M., 1997. Domestication of the white grouper, *Epinephelus aeneus*: 1. Growth and reproduction. Aquaculture 156, 305–316.

Hastings, P.A., 1981. Gonad morphology and sex succession in the protogynous hermaphrodite *Hemanthias vivanus* (Jordan and Swain). J. Fish Biol. 18, 443–454.

Hastings, P.A., 1989. Protogynous hermaphroditism in *Paralabrax maculatofasciatus* (Pisces: Serranidae). Copeia 1989, 184–188.
Hattori, A., 1991. Socially controlled growth and size-dependent sex change in the anemonefish *Amphiprion frenatus* in Okinawa, Japan. Jpn. J. Ichthyol. 38, 165–178.
Hattori, A., 1994. Inter-group movement and mate acquisition tactics of the protandrous anemonefish, *Amphiprion clarkii*, on a Coral Reef, Okinawa. Jpn. J. Ichthyol. 41, 159–165.
Hattori, A., Yanagisawa, Y., 1991a. Life-history pathways in relation to gonadal sex differentiation in the anemonefish, *Amphiprion clarkii*, in temperate waters of Japan. Environ. Biol. Fishes 31, 139–155.
Hattori, A., Yanagisawa, Y., 1991b. Sex change of the anemonefish *Amphiprion clarkii* in a habitat of high host density a removal study. Jpn. J. Ecol. 41, 1–8.
Hawkins, J.R., 1994. Sex determination. Hum. Mol. Genet. 3, 1463–1467.
Heppel, S.A., Denslow, N.D., Folmar, L.C., Sullivan, C.V., 1995a. Universal vertebrate vitellogenin antibodies. Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish, Fish Symposium 95, Austin, TX (USA), p. 363.
Heppel, S.A., Denslow, N.D., Folmar, L.C., Sullivan, C.V., 1995b. Universal assay of vitellogenin as a biomarker for environmental estrogens. Environ. Health Perspect. 103, 9–15.
Herman, R.L., Kincaid, H.L., 1986. Gonadal dysgenesis in rainbow trout *Salmo gairdneri*. Dis. Aquat. Org. 1, 227–228.
Herman, R.L., Kincaid, H.L., 1991. Effects of orally administered steroids on lake trout and Atlantic salmon. Prog. Fish-Cult. 53, 157–161.
Herrera, G., Padilla, C., Claramunt, G., Pizarro, P., Garland, D., 1991. Synchronous hermaphroditism of the intersex type in the spanish sardine *Sardinops sagax* Jenyns 1842. Rev. Biol. Mar. 26, 81–89.
Hewitt, L.M., Munkittrick, K.R., Van Der Kraak, G.J., Scott, I.M., Schleen, L.P., Servos, M.R., 1998. Hepatic mixed function oxygenase activity and vitellogenin induction in fish following a treatment of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM). Can. J. Fish. Aquat. Sci. 55, 2078–2086.
Hikita, T., Hashimoto, S., 1978. A hermaphroditic chum salmon, *Oncorhynchus keta*, from the Chitose River, with an example of its self fertilization. Sci. Rep. Hokkaido Salmon Hatchery 32, 61–64.
Himick, B.A., Peter, R.E., 1995. Bombesin-like immunoreactivity in the forebrain and pituitary and regulation of anterior pituitary hormone release by bombesin in goldfish. Neuroendocrinology 61, 365–376.
Hinegardner, R., Rosen, D.E., 1972. Cellular DNA content and the evolution of teleostean fishes. Am. Nat. 106, 621–644.
Hioki, S., Tanaka, Y., Suzuki, K., 1995. Reproductive behavior, eggs, larvae, and sexuality of two angelfishes, *Genicanthus watanabei* and *G. bellus*, in an aquarium. J. Sch. Mar. Sci. Technol. Tokai Univ. 40, 151–171.
Hiott, A.E., Phelps, R.P., 1993. Effects of initial age and size on sex reversal of *Oreochromis niloticus* fry using methyltestosterone. Aquaculture 112, 301–308.
Hirose, K., Nagahama, Y., Adachi, S., Wakabayashi, K., 1983. Changes in serum concentrations of gonadotropin, 17 alpha-hydroxyprogesterone and 17alpha, 20beta-dihydroxy-4-pregnen-3-one during synthetic LH-RH-induced final oocyte maturation and ovulation in the ayu *Plecoglossus altivelis*. Bull. Jpn. Soc. Sci. Fish. 49, 1165–1169.
Hoar, W.S., Nagahama, Y., 1978. The cellular sources of sex steroids in teleost gonads. Ann. Biol. Anim., Biochim., Biophys. 18, 893–898.
Hoerstgen Schwark, G., 1993. Production of homozygous diploid zebra fish *Brachydanio rerio*. Aquaculture 112, 25–37.
Hoffman, S.G., 1983. Sex-related foraging behavior in sequentially hermaphroditic hogfishes (*Bodianus* spp.). Ecology 64, 798–808.
Hogan, J.C., 1973. The fate and fine structure of primordial gem cells in the teleost, *Oryzias latipes*. J. Cell Biol. 59, 146.
Hollebecq, M.G., Chourrout, D., Wohlfarth, G., Billard, R., 1986. Diploid gynogenesis induced by heat shocks after activation with UV-irradiated sperm in common carp. Aquaculture 54, 69–76.
Holmgren, K., 1996. Effect of water temperature and growth variation on the sex ratio of experimentally reared eels. Ecol. Freshwater Fish 5, 203–212.
Hong, W., Zhang, Q., Zheng, J., Huang, Z., 1991. Studies on gonadal development and sex inversion of yellowfin seabream (*Sparus latus*). J. Oceanogr. Taiwan Strait 10, 221–228.

Honma, Y., 1980. A synchronous (balanced) hermaphroditism in a chum salmon, *Oncorhynchus keta*, from the Sea of Japan. Annu. Rep. Sado Mar. Biol. Stn. 10, 17–22.

Hopkins, K.D., Shelton, W.L., Engle, C.R., 1979. Estrogen sex-reversal of *Tilapia aurea*. Aquaculture 18, 263–268.

Hornaday, K., Alexander, S., Breden, F., 1994. Absence of repetitive DNA sequences associated with sex chromosomes in natural populations of the Trinidad guppy (*Poecilia reticulata*). J. Mol. Evol. 39, 431–433.

Horvath, M.L., Grimes, C.B., Huntsman, G.R., 1990. Growth, mortality, reproduction and feeding of knobbed porgy, *Calamus nodosus*, along the southeastern United States coast. Bull. Mar. Sci. 46, 677–687.

Hostache, G., Pascal, M., Tessier, C., 1995. Effects of the incubation temperature on the male: female ratio in atipa *Hoplosternum littorale* Hancock (1828). Can. J. Zool. 73, 1239–1246.

Houde, A.E., 1992. Sex-linked heritability of a sexually selected character in a natural population of *Poecilia reticulata* (Pisces: Poeciliidae) (guppies). Heredity 69, 229–235.

Hourigan, T.F., Kelley, C.D., 1985. Histology of the gonads and observations on the social behavior of the Caribbean angelfish *Holacanthus tricolor*. Mar. Biol. 88, 311–322.

Hourigan, T.F., Nakamura, M., Nagahama, Y., Yamauchi, K., Grau, E.G., 1991. Histology, ultrastructure, and in vitro steroidogenesis of the testes of two male phenotypes of the protogynous fish, *Thalassoma duperrey* (Labridae). Gen. Comp. Endocrinol. 83, 193–217.

Howe, J.C., 1996. Sexual dimorphism in the bicolor bass, *Anthias bicolor* (Pisces: Serranidae), with comments on stomach contents. J. Aquacult. Aquat. Sci. 8, 16–18.

Howell, W., Black, D., Bortone, S., 1980. Abnormal expression of secondary sex characters in a population of mosquitofish, *Gambusia affinis* holbrooki: evidence for environmentally-induced masculinization. Copeia 1980, 676–681.

Howell, B.R., Baynes, S.M., Thompson, D., 1995. Progress towards the identification of the sex-determining mechanism of the sole, *Solea solea* (L.), by the induction of diploid gynogenesis. Aquacult. Res. 26, 135–140.

Huang, C.C., Lo, C.T., Chang, K.H., 1974. Sex reversal in one sparid fish *Crysophrys major* (Perciformes, Sparidae). Inst. Zool. Acad. Sin. 13, 55–60.

Huang, Y.S., Schmitz, M., Le Belle, N., Chang, C.F., Querat, B., Dufour, S., 1997. Androgens stimulate gonadotropin-II beta-subunit in eel pituitary cells in vitro. Mol. Cell. Endocrinol. 131, 157–166.

Hubbs, C.L., Hubbs, L.C., 1932. Apparent parthenogenesis in nature, in a form of fish of hybrid origin. Science 76, 628–630.

Hulata, G., Wohlfarth, G., Rothbard, S., 1983. Progeny-testing selection of tilapia broodstocks producing all-male hybrid progenies—preliminary results. Aquaculture 33, 1–4.

Hunsinger, R.N., Byram, B.R., Howell, W.M., 1988. Unchanged gonadal morphology of mosquitofish masculinized by exposure to degrade plant sterols. J. Fish Biol. 32, 795–796.

Hunter, G.A., Donaldson, E.M., 1983. Hormonal sex control and its application to fish culture. Fish Physiol. 9, 223–303.

Hunter, G.A., Donaldson, E.M., Goetz, F.W., Edgell, P.R., 1982. Production of all-female and sterile coho salmon, and experimental evidence for male heterogamety. Trans. Am. Fish. Soc. 111, 367–372.

Hunter, G.A., Donaldson, E.M., Stoss, J., Baker, I., 1983. Production of monosex female groups of chinook salmon (*Oncorhynchus tshawytscha*) by the fertilization of normal ova with sperm from sex-reversed females. Aquaculture 33, 1–4.

Husebye, H., Lund, S., Moeller, M., Sunde, A., Krokan, H.E., 1994. A Bkm-related DNA sequence gives individual DNA fingerprints in turbot (*Scophthalmus maximus*), but neither Bkm-related, human SRY or human ZFY probes detect genetic sex differences. Comp. Biochem. Physiol., B 107B, 69–73.

Hussain, M.G., Penman, D.J., McAndrew, B.J., 1996. Effects of triploidy on sexual maturation and reproduction in Nile tilapia, *Oreochromis niloticus* L. ICLARM Conference Proceedings, Makati City (Philippines). ICLARM, Makati City, Philippines, pp. 320–325.

Hussain, M.G., Penman, D.J., McAndrew, B.J., 1998. Production of heterozygous and homozygous clones in Nile tilapia. Aquacult. Int. 6, 197–205.

Ikeuchi, T., Todo, T., Kobayashi, T., Nagahama, Y., 1999. cDNA cloning of a novel androgen receptor subtype. J. Biol. Chem. 274, 25205–25209.

Ishii, K., Yabu, H., 1985. Chromosomes in three species of Gadidae (Pisces). Bull. Jpn. Soc. Sci. Fish. 51, 25–28.

Ito, M., Ishikawa, M., Suzuki, S., Takamatsu, N., Shiba, T., 1995. A rainbow trout SRY-type gene expressed in pituitary glands. FEBS Lett. 377, 37–40.
Ito, M., Masuda, A., Yumoto, K., Otomoto, A., Takahashi, N., Takamatsu, N., 1998. cDNA cloning of a new member of the FTZ-F1 subfamily from a rainbow trout. Biochim. Biophys. Acta 1395, 271–274.
Iturra, P., Medrano, J.F., Bagley, M., Lam, N., Vergara, N., Marin, J.C., 1998. Identification of sex chromosome molecular markers using RAPDs and fluorescent in situ hybridization in rainbow trout. Genetica 101, 209–213.
Iturra, P., et al., 2001. Characterization of sex chromosomes in rainbow trout and coho salmon using fluorescent in situ hybridization (FISH). Genetica 111, 125–131.
Iwamatsu, T., Uwa, H., Inden, A., Hirata, K., 1984. Experiments on interspecific hydribization between *Oryzias latipes* and *Oryzias celebensis*. Zool. Sci. 1, 653–663.
Iwasa, Y., 1991. Sex change evolution and cost of reproduction. Behav. Ecol. 2, 56–68.
Jafri, S.I.H., Ensor, D.M., 1979. Occurrence of an intersex condition in the roach *Rutilus rutilus* (L). J. Fish Biol. 14, 547–549.
Jalabert, B., Billard, R., Chevassus, B., 1975. Preliminary experiments on sex control in trout: production of sterile fishes and self fertilization of hermaphrodites. Ann. Biol. Anim., Biochim., Biophys. 15, 19–28.
Janssen, P.A.H., Lambert, J.G.D., Vethaak, A.D., Goos, H.J.T., 1997. Environmental pollution caused elevated concentrations of oestradiol and vitellogenin in the female flounder, *Platichthys flesus* (L.). Aquat. Toxicol. 39, 195–214.
Jellyman, D.J., 1976. Hermaphrodite European perch *Perca fluviatilis* L. N. Z. J. Mar. Freshwater Res. 10, 721–723.
Jensen, G.L., Shelton, W.L., 1979. Effects of estrogens on *Tilapia aurea*: implications for production of monosex genetic male Tilapia. Aquaculture 16, 233–242.
Jensen, G.L., Shelton, W.L., Yang, S.L., Wilken, L.O., 1983. Sex reversal of gynogenetic grass carp by implantation of methyltestosterone. Trans. Am. Fish. Soc. 112, 79–85.
Jessy, D., Varghese, T.J., 1987. Hormonal sex control in *Betta splendens* regan and *Xiphophorus helleri* Heckel. The First Indian Fisheries Forum, Proceedings, Mangalore, India, 123–124.
Jiang, J.-Q., Kobayashi, T., Ge, W., Kobayashi, H., Tanaka, M., Okamoto, M., Nonaka, Y., Nagahama, Y., 1996. Fish testicular 11 beat-hydroxylase: cDNA cloning and mRNA expression during spermatogenesis. FEBS Lett. 397, 250–252.
Jobling, S., Sumpter, J.P., 1993. Detergent components in sewage effluent are weakly oestrogenic to fish: an in vitro study using rainbow trout (*Oncorhynchus mykiss*) hepatocytes. Aquat. Toxicol. 27, 361–372.
Jobling, S., Reynolds, T., White, R., Parker, M.G., Sumpter, J.P., 1995. A variety of environmentally-persistent chemicals, including some phthalate plasticizers, are weakly oestrogenic. Environ. Health Perspect. 103, 582–587.
Jobling, S., Sheahan, D., Osborne, J.A., Matthiessen, P., Sumpter, J.P., Ashfield, L.A., Pottinger, T.G., 1996. Exposure of female juvenile rainbow trout to alkylphenolic compounds results in modifications to growth and ovosomatic index. Environ. Toxicol. Chem. 15, 194–202.
Jobling, S., Nolan, M., Tyler, C.R., Brighty, G., Sumpter, J.P., 1998. Widespread sexual disruption in wild fish. Environ. Sci. Technol. 32, 2498–2506.
Johnson, A.K., Thomas, P., 1995. Seasonal changes in gonadal histology and sex steroid hormone levels in the protogynous hermaphrodite, *Epinephelus morio.* Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish, The University Of Texas At Austin, Fish Symposium 95, Austin, TX (USA), p. 234.
Johnson, A.K., Thomas, P., Wilson Jr., R.R., 1998. Seasonal cycles of gonadal development and plasma sex steroid levels in *Epinephelus morio*, a protogynous grouper in the eastern Gulf of Mexico. J. Fish Biol. 52, 502–518.
Johnston, P.M., 1951. The embryonic history of the germ cells of the largemouth black bass, *Micropterus salmoides salmoides* (Lacepede). J. Morphol. 88, 471–542.
Johnstone, R., Youngson, A.F., 1984. The progeny of sex-inverted female *Atlantic salmon* (Salmo salar L.). Aquaculture 37, 179–182.
Johnstone, R., Simpson, T.H., Youngson, A.F., 1978. Sex reversal in salmonid culture. Aquaculture 13, 115–134.

Johnstone, R., Simpson, T., Walker, A., 1979a. Sex reversal in salmonid culture: Part 3. The production and performance of all-female populations of brook trout. Aquaculture 18, 241–252.
Johnstone, R., Simpson, T.H., Youngson, A.F., Whitehead, C., 1979b. Sex reversal in salmonid culture: Part 2. The progeny of sex-reversed rainbow trout. Aquaculture 18, 13–19.
Jones, G.P., 1980a. Growth and reproduction in the protogynous hermaphrodite *Pseudolabrus celidotus* (Pisces: Labridae) in New Zealand. Copeia 1980, 660–675.
Jones, G.P., 1980b. Contribution to the biology of the redbanded perch, *Ellerkeldia huntii* (Hector), with a discussion on hermaphroditism. J. Fish Biol. 17, 197–207.
Jones, J.C., Reynolds, J.D., 1997. Effects of pollution on reproductive behaviour of fishes. Rev. Fish Biol. Fish. 7, 463–491.
Jory, D.E., Iversen, E.S., 1989. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (South Florida), black, red, and Nassau groupers. Biol. Rep. U.S. Fish Wildl. Serv. 1–30.
Kacser, V.T., Burns, J.A., 1981. The molecular basis of dominance. Genetics 98, 639–666.
Kagawa, H., Young, G., Adachi, S., Nagahama, Y., 1982. Estradiol-17 beta production in amago salmon (*Oncorhynchus rhodurus*) ovarian follicles: role of the thecal and granulosa cells. Gen. Comp. Endocrinol. 47, 440–448.
Kagawa, H., Young, G., Nagahama, Y., 1984. In vitro estradiol-17 beta and testosterone production by ovarian follicles of the goldfish, *Carassius auratus*. Gen. Comp. Endocrinol. 54, 139–143.
Kagawa, H., Moriyama, S., Kawauchi, H., 1995. Immunocytochemical localization of IGF-I in the ovary of the red seabream, *Pagrus major*. Gen. Comp. Endocrinol. 99, 307–315.
Kagwade, P.V., 1976. Sexuality in *Polydactylus indicus* (Shaw). Indian J. Fish. 21, 323–329.
Kakimoto, Y., Aida, S., Arai, K., Suzuki, R., 1994a. Production of gynogenetic diploids by temperature and pressure treatments and sex reversal by immersion in methyltestosterone in marbled sole *Limanda yokohamae*. Appl. Biol. Sci./Seibutsu Seisangaku Kenkyu 33, 113–124.
Kakimoto, Y., Aida, S., Arai, K., Suzuki, R., 1994b. Induction of gynogenetic diploids in ocellated puffer *Takifugu rubripes* by cold and heat treatments. Appl. Biol. Sci./Seibutsu Seisangaku Kenkyu 33, 103–112.
Kallman, K.D., 1965. Genetics and geography of sex determination in the Poeciliid fish, *Xiphophorus maculatus*. Zoologica 50, 151–190.
Kallman, K.D., 1984a. Sex ratio and the genetics of sex determination in swordtails, *Xiphophorus Poeciliidae*. Genetics 107, s54.
Kallman, K.D., 1984b. A new look at sex determination in poeciliid fishes. Evol. Genet. Fishes, 95–171.
Kallman, K.D., Bao, I.Y., 1987. Female heterogamety in the swordtail, *Xiphophorus alvarezi* Rosen (Pisces, Poeciliidae), with comments on a natural polymorphism affecting sword coloration. J. Exp. Zool. 243, 93–102.
Kallman, K.D., Borkoski, V., 1978. A sex-linked gene controlling the onset of sexual maturity in female and male platyfish (*Xiphophorus maculatus*), fecundity in females and adult size in males. Genetics 89, 79–119.
Kanamori, A., Nagahama, Y., 1988. Involvement of 3′,5′-cyclic adenosine monophosphate in the control of follicular steroidogenesis of amago salmon (*Oncorhynchus rhodurus*). Gen. Comp. Endocrinol. 72, 39–53.
Kanamori, A., Kitajima, K., Inoue, Y., Inoue, S., Hoshino, K., Matsuoka, E., Sato, A., Satsumi, K., 1993. Immunochemical method as early diagnosis for distinguishing salmonid fish female and male prior to genital maturation by use of a minute amount of blood collected from a single piece of gill filament. Bull. Jpn. Soc. Sci. Fish./Nippon Suisan Gakkaishi 59, 593–600.
Kanda, H., Kojima, M., Miyamoto, N., Ito, M., Takamatsu, N., Yamashita, S., Shiba, T., 1998. Rainbow trout Sox24, a novel member of the Sox family, is a transcriptional regulator during oogenesis. Gene 211, 251–257.
Katayama, M., 1978. The anthiine fish, *Pseudanthias taeniatus*, from Hachijo Island and the Coast of Izu, Japan. Jpn. J. Ichthyol. 25, 216–218.
Katsu, Y., Yamashita, M., Nagahama, Y., 1997. Isolation and characterization of goldfish Y box protein, a germ-cell-specific RNA-binding protein. Eur. J. Biochem. 249, 854–864.
Kavumpurath, S., Pandian, T.J., 1990. Induction of Triploidy in the Zebrafish *Brachydanio rerio* Hamilton. Aquacult. Fish. Manage. 21, 299–306.
Kavumpurath, S., Pandian, T.J., 1992a. Production of YY male in the guppy *Poecilia reticulata* by endocrine sex reversal and progeny testing. Asian Fish. Sci. 5, 265–276.

Kavumpurath, S., Pandian, T.J., 1992b. The development of all-male sterile triploid fighting fish (*Betta splendens* Regan) by integrating hormonal sex reversal of broodstock and chromosome-set manipulation. Isr. J. Aquacult. 44, 111–119.

Kavumpurath, S., Pandian, T.J., 1993a. Masculinization of *Poecilia reticulata* by dietary administration of synthetic or natural androgen to gravid females. Aquaculture 116, 83–89.

Kavumpurath, S., Pandian, T.J., 1993b. Determination of labile period and critical dose for sex reversal by oral administration of estrogens in *Betta splendens* Regan. Indian J. Exp. Biol. 31, 16–20.

Kavumpurath, S., Pandian, T.J., 1994a. Masculinization of fighting fish, *Betta splendens* Regan, using synthetic or natural androgens. Aquacult. Fish. Manage. 25, 373–381.

Kavumpurath, S., Pandian, T.J., 1994b. Induction of heterozygous and homozygous diploid gynogenesis in *Betta splendens* (Regan) using hydrostatic pressure. Aquacult. Fish. Manage. 25, 133–142.

Kawahara, T., Yamashita, I., 2000. Estrogen-independent ovary formation in the Medaka fish, *Oryzias latipes*. Zool. Sci. 17, 65–68.

Kawamura, K.O., 1998. Sex determination system of the rosy bitterling, *Rhodeus ocellatus ocellatus*. Environ. Biol. Fishes 52, 251–260.

Kelly, M.J., Lagrange, A.H., Wagner, E.J., Rønnekleiv, O.K., 1999. Rapid effects of estrogen to modulate G protein-coupled receptors via activation of protein kinase A and protein kinase C pathways. Steroids 64, 64–79.

Kent, J., Wheatley, S., Andrews, J., Sinclair, A., Koopman, P., 1996. A male-specific role for SOX9 in vertebrate sex determination. Development 122, 2813–2822.

Khan, M.N., Renaud, R.L., Leatherland, J.F., 1997. Metabolism of estrogens and androgens by embryonic tissues of Arctic charr, *Salvelinus alpinus*. Gen. Comp. Endocrinol. 107, 118–127.

Khoo, G., Lim, T.M., Chan, W.K., Phang, V.P.E., 1999. Sex-linkage of the black caudal-peduncle and red tail genes in the tuxedo strain of the guppy, *Poecilia reticulata*. Zool. Sci. 16, 629–638.

Khuda-Bukhsh, A.R., 1979a. Chromosomes in three species of fishes, *Aplocheilus panchax* (Cyprinodontidae), *Lates calcerifer* (Percidae) and *Gadusia chapra* (Clupeidae). Caryologia 32, 161–169.

Khuda-Bukhsh, A.R., 1979b. Karyology of two species of hillstream fish, *Barilius bendelisis* and *Rasbora daniconius* (fam: Cyprinidae). Curr. Sci. 48, 793–794.

Khuda-Bukhsh, A.R., Chakrabarti, J., 1999. Indication of the sex chromosome pair bearing Ag–NORs in a brackish water fish, *Scatophagus argus* showing male heterogamety. Indian J. Exp. Biol. 37, 793–797.

Khuda Bukhsh, A.R., Datta, S., 1997. Sex-specific difference in NOR-location on metaphase chromosomes of mosquito fish, *Aplocheilus panchax* (Cyprinodontidae). Indian J. Exp. Biol. 35, 1111–1114.

Khuda-Bukhsh, A.R., Manna, G.K., 1977. Cited in Rishi, K.K. Current status of fish cytogenetics. In: Das and Jhingram (Eds.), Fish Genetics in India, Today and Tomorrow's Printers and Publishers, New Delhi, India. Geobios 4, p. 49.

Khuda-Bukhsh, A.R., Gupta, S.K., Goswami, S., 1980. Karyotypic studies in *Garra lamta* and *Mystus cavassius* (Pisces). Proc. Indian Acad. Sci., Anim. Sci. 89, 557–562.

Khuda Bukhsh, A.R., Rahman, A., Chanda, T., 1992. A study of chromosomes in three species of hillstream fish belonging to the genus *Barilius* Cyprinidae Pisces. Proc. Acad. Sci. India B 62, 199–203.

Kim, D.S., Kim, I.B., Huh, H.T., Park, I.S., 1988. Cytogenetic analysis of catfish, *Silurus asotus* (Teleostomi: Siluriformes). Contrib. Inst. Mar. Sci. Natl. Fish. Univ. Pusan 20, 31–35.

Kim, D.S., Kim, J.H., Jo, J.Y., Moon, Y.B., Cho, K.C., 1993. Induction of gynogenetic diploid in *Paralichthys olivaceus*. Korean J. Genet. 15, 179–186.

Kim, D.S., Nam, Y.K., Jo, J.Y., 1997a. Effect of oestradiol-17 beta immersion treatments on sex reversal of mud loach, *Misgurnus mizolepis* (Guenther). Aquacult. Res. 28, 941–946.

Kim, Y., Kim, W.J., Baek, H.J., Kim, K.K., Bang, I.C., Han, C.H., 1997b. Immunological characteristics of the vitellogenin induced by Estradiol-17 beta in male spotted flounder, *Verasper variegatus*. J. Korean Fish. Soc. 30, 480–487.

Kime, D.E., 1993. "Classical" and "non-classical" reproductive steroids in fish. Rev. Fish Biol. Fish. 3, 160–180.

Kime, D.E., 1995. The effects of pollution on reproduction in fish. Rev. Fish Biol. Fish. 5, 52–96.

Kime, D.E., 1998. Endocrine Disruption in Fish. Klewar Academic Publishers, Boston, 416 pp.

Kime, D.E., Hyder, M., 1983. The effect of temperature and gonadotropin on testicular steroidogenesis in *Sarotherodon* (tilapia) *mossambicus* in vitro. Gen. Comp. Endocrinol. 50, 105–115.

Kime, D.E., Manning, N.J., 1986. Maturational and temperature effects on steroid hormone production by testes of the carp, *Cyprinus carpio*. Aquaculture 54, 44–55.

Kime, D.E., Lone, K.P., Al Marzouk, A., 1991. Seasonal changes in serum steroid hormones in a protandrous teleost the sobaity *Sparidentex hasta* Valenciennes. J. Fish Biol. 39, 745–754.

Kirpichnikov, V.S., 1981. Genetic Basis of Fish Selection. Springer-Verlag, Berlin.

Kitano, T., Takamune, K., Kobayashi, T., Nagahama, Y., Abe, S.-I., 1999. Suppression of *P*450 aromatase gene expression in sex-reversed males produced by rearing genetically female larvae at a high water temperature during a period of sex differentiation in the Japanese flounder (*Paralichthys olivaceus*). J. Mol. Endocrinol. 23, 167–176.

Kitano, T., Takamune, K., Nagahama, Y., Abe, S.-I., 2000. Aromatase inhibitor and 17alpha-methyltestosterone cause sex-reversal from genetical females to phenotypic males and suppression of *P*450 aromatase gene expression in Japanese flounder (*Paralichthys olivaceus*). Mol. Reprod. Dev. 56, 1–5.

Klinkhardt, M.B., 1993. Cytogenetics of *Platichthys flesus* and *Limanda limanda* (Pleuronectidae, Teleostei) and caryoevolution of Pleuronectiformes. Z. Fischkd. 2, 66–67.

Klinkhardt, M.B., 1994. Karyotypic divergence between species of Gadidae (Pisces, gadiformes). Cytobios 77, 3–11.

Klinkhardt, M.B., Buuk, B., 1990a. Karyological studies in several species of freshwater fishes from brackish coastal waters of the southwestern baltic sea III. The three-spined stickleback *Gasterosteus-Aculeatus* Linnaeus 1758 and the nine-spined stickleback *Pungitius-Pungitius* Linnaeus 1758. Zool. Anz. 225, 341–352.

Klinkhardt, M.B., Buuk, B., 1990b. The chromosomes of carp (*Cyprinus carpio*). Z. Binnenfisch. DDR. 37, 188–191.

Klinkhardt, M.B., Buuk, B., 1991. Karyological studies in several species of freshwater fishes from brackish coastal waters of South Western baltic IV. The perch *Perca fluviatilis* Linnaeus 1758. Zool. Anz. 227, 38–43.

Klotz, A.V., Stegeman, J.J., Woodin, B.R., Snowberger, E.A., Thomas, P.E., Walsh, C., 1986. Cytochrome *P*-450 isozymes from the marine teleost *Stenotomus chrysops*: their roles in steroid hydroxylation and the influence of cytochrome *b*5. Arch. Biochem. Biophys. 249, 326–328.

Kobayashi, H., 1976. A cytological study on the maturational division in the oogenic process of the triploid ginbuna (*Carrassius auratus* Langsdorfii). Jpn. J. Ichthyol. 22, 234–240.

Kobayashi, M., Stacey, N., 1993. Prostaglandin-induced female spawning behavior in goldfish *Carassius auratus* appears independent of ovarian influence. Horm. Behav. 27, 38–55.

Kobayashi, K., Suzuki, K., 1990. Gonadogenesis and sex succession in the protogynous wrasse, *Cirrhilabrus temminckii*, in Suruga Bay, central Japan. Jpn. J. Ichthyol. 37, 256–264.

Kobayashi, K., Suzuki, K., 1992. Hermaphroditism and sexual function in *Cirrhitichthys aureus* and the other Japanese hawkfishes (Cirrhitidae: Teleostei). Jpn. J. Ichthyol. 38, 397–410.

Kobayashi, M., Aida, K., Hanyu, I., 1987. Hormone changes during ovulation and effects of steroid hormones on plasma gonadotropin levels and ovulation in goldfish. Gen. Comp. Endocrinol. 67, 24–32.

Kobayashi, M., Aida, K., Hanyu, I., 1988. Hormone changes during the ovulatory cycle in goldfish. Gen. Comp. Endocrinol. 69, 301–307.

Kobayashi, M., Aida, K., Stacey, N.E., 1991. Induction of testis development by implantation of 11-ketotestosterone in female goldfish. Zool. Sci. 8, 389–393.

Kobayashi, K., Suzuki, K., Shiobara, Y., 1993a. Reproduction and hermaphroditism in *Parapercis snyderi* (Teleostei, Parapercidae) in Suruga Bay, Central Japan. J. Fac. Mar. Sci. Technol., Tokai Univ. 35, 149–168.

Kobayashi, T., Sakai, N., Fushiki, S., Nagahama, Y., Amano, M., Aida, K., 1993b. Testicular development and changes in the levels of reproductive hormones in triploid male rainbow trout. Bull. Jpn. Soc. Sci. Fish./ Nippon Suisan Gakkaishi 59, 981–989.

Kobayashi, T., Ide, A., Hiasa, T., Fushiki, S., Ueno, K., 1994. Production of cloned amago salmon *Oncorhynchus rhodurus*. Fish. Sci. 60, 275–281.

Kobayashi, D., Tanaka, M., Fukada, S., Nagahama, Y., 1996a. Steroidogenesis in the ovarian follicles of the medaka (*Oryzias latipes*) during vitellogenesis and oocyte maturation. Zool. Sci. 13, 921–927.

Kobayashi, T., Chang, X.-T., Nakamura, M., Kijura, H., Nagahama, Y., 1996b. Fish 3 beta-hydroxysteroid dehydrogenase/Delta 5-delta 4 isomerase: antibody production and their use for the immunohistochemical detection of fish steroidogenic tissues. Zool. Sci. 13, 909–914.

Kobayashi, T., Nakamura, M., Kajiura-Kobayashi, H., Young, G., Nagahama, Y., 1998. Immunolocalization of

steroidogenic enzymes (*P*450scc, *P*450c17, *P*450arom, and 3beta-HSD) in immature and mature testes of rainbow trout (*Oncorhynchus mykiss*). Cell Tissue Res. 292, 573–577.

Koehler, M.R., Neuhaus, D., Engel, W., Schartl, M., Schmid, M., 1995. Evidence for an unusual ZW/ZW′/ZZ sex-chromosome system in *Scardinius erythrophthalmus* (Pisces, Cyprinidae), as detected by cytogenetic and H–Y antigen analyses. Cytogenet. Cell Genet. 71, 356–362.

Koenig, C.C., Abel, D.C., Klingensmith, C.W., Maddock, M.B., 1982. Usefulness of the Self-Fertilizing Cyprinodontid Fish, *Rivulus marmoratus* as an Experimental Animal in Studies Involving Carcinogenesis, Teratogenesis and Mutagenesis CC/GMBL, Charleston, SC, USA, 143 pp.

Kohne, D.E., Levison, S.A., Byers, M.J., 1977. Room temperature method for increasing the rate of DNA reassociation by many thousandfold: the phenol emulsion reassociation technique. Biochemistry 24, 5329–5341.

Kolluru, G.R., Reznick, D.N., 1996. Genetic and social control of male maturation in *Phallichthys quadripunctatus* (Pisces: Poeciliidae). J. Evol. Biol. 9, 695–715.

Koltes, K.H., 1993. Aspects of the reproductive biology and social structure of the stoplight parrotfish *Sparisoma viride* at Grand Turk, Turks and Caicos Islands B.W.I. Bull. Mar. Sci. 52, 792–805.

Komen, J., Bongers, A.B.J., Richter, C.J.J., van Muiswinkel, W.B., Huisman, E.A., 1991. Gynogenesis in common carp (*Cyprinus carpio* L.): 2. The production of homozygous gynogenetic clones and F sub(1) hybrids. Aquaculture 92, 127–142.

Komen, J., de Boer, P., Richter, C.J.J., 1992a. Male sex reversal in gynogenetic XX females of common carp *Cyprinus carpio* L. by a recessive mutation in a sex-determining gene. J. Hered. 83, 431–434.

Komen, J., Yamashita, M., Nagahama, Y., 1992b. Testicular development induced by a recessive mutation during gonadal differentiation of female common carp *Cyprinus carpio* L. Dev., Growth Differ. 34, 535–544.

Komen, J., Wiegertijes, G.F., Ginneken, V., Eding, E.H., Richter, C.J.J., 1992c. Gynogenesis in common carp (*Cyprinus carpio* L.): 3. The effects of inbreeding on gonadal development of heterozygous and homozygous gynogenetic offspring. Aquaculture 104, 1–2.

Komen, J., Eding, E.H., Bongers, A.B.J., Richter, C.J.J., 1993. Gynogenesis in common carp (*Cyprinus carpio*): 4. Growth, phenotypic variation and gonad differentiation in normal and methyltestosterone-treated homozygous clones and F sub(1) hybrids. Aquaculture 111, 271–280.

Komen, J., Lambert, J.G.D., Richter, C.J.J., Goos, H., 1995. Endocrine control of sex differentiation in XX female, and in XY and XX male common carp (*Cyprinus carpio*, L.). Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish. Fish Symposium 95, Austin, TX (USA), p. 383.

Koopman, P., 1999. Sry and Sox9: mammalian testis-determining genes. Cell. Mol. Life Sci. 55, 839–856.

Koopman, P., 2001. Sry, Sox9 and mammalian sex determination. EXS 91, 25–56.

Koopman, P., Gubbay, J., Vivian, N., Goodfellow, P., Lovell-Badge, R., 1991. Male development of chromosomally female mice transgenic for Sry. Nature 351, 117–121.

Koulish, S., Kramer, C.R., 1989. Human chorionic gonadotropin (hCG) induces gonad reversal in a protogynous fish, the bluehead wrasse, *Thalassoma bifasciatum* (Teleostei, Labridae). J. Exp. Zool. 252, 156–168.

Kovacs, T.G., Gibbsons, J.S., Tremblay, L.A., O'Connor, B.I., Martel, P.H., Voss, R.H., 1995. The effects of a secondary-treated bleached kraft mill effluent on aquatic organisms as assessed by short-term and long-term laboratory tests. Ecotoxicol. Environ. Saf. 31, 7–22.

Koyano, S., Ito, M., Takamatsu, N., Takiguchi, S., Shiba, T., 1997. The *Xenopus* Sox3 gene expressed in oocytes of early stages. Gene 188, 101–107.

Kraak, S.B.M., de Looze, E.M.A., 1993. A new hypothesis on the evolution of sex determination in vertebrates: big females ZW, big males XY. Neth. J. Zool. 43, 260–273.

Kraljevic, M., Dulcic, J., Pallaoro, A., Cetinic, P., Jug Dujakovic, J., 1995. Sexual maturation, age and growth of striped sea bream, *Lithognathus mormyrus* L., on the eastern coast of the Adriatic sea. J. Appl. Ichthyol. 11, 1–8.

Kramer, C.R., Imbriano, M.A., 1997. Neuropeptide Y (NPY) induces gonad reversal in the protogynous bluehead wrasse, *Thalassoma bifasciatum* (Teleostei: Labridae). J. Exp. Zool. 279, 133–144.

Kramer, C.R., Kallman, K.D., 1985. Sex differentiation of somatic tissue in the unsexualised gonad primordia of the embryos of three species of poeciliid fish. J. Anat. 140, 269–277.

Kramer, C.R., Koulish, S., Bertacchi, P.L., 1988. The effects of testosterone implants on ovarian morphology in the bluehead wrasse, *Thalassoma bifasciatum* (Bloch) (Teleostei: Labridae). J. Fish Biol. 32, 397–407.

Kramer, C.R., Caddell, M.T., Bubenheimer-Livolsi, L., 1993. sGnRH-A ((D-Arg super(6), Pro super(9), NEt-) LHRH) in combination with domperidone induces gonad reversal in a protogynous fish, the bluehead wrasse, *Thalassoma bifasciatum*). J. Fish Biol. 42, 185–195.

Krisfalusi, M., Cloud, J.G., 1999. Gonadal sex reversal in triploid rainbow trout (*Oncorhynchus mykiss*). J. Exp. Zool. 284, 466–472.

Krisfalusi, M., Eroschenko, V.P., Cloud, J.G., 1998. Exposure of juvenile rainbow trout *Oncorhynchus mykiss* to methoxychlor results in a dose-dependent decrease in growth and survival but does not alter male sexual differentiation. Bull. Environ. Contam. Toxicol. 60, 659–666.

Krishnaja, A.P., Rege, M.S., 1979. Genetic studies on two species of the Indian carp *Labeo* and their fertile F1 and F2 hybrids. Indian J. Exp. Biol. 17, 253–257.

Krishnaja, A.P., Rege, M.S., 1983. A cytogenetic study on the *Gambusia affinis* population from India. Cytologia 48, 47–49.

Kroon, F.J., Liley, N.R., 2000. The role of steroid hormones in protogynous sex change in the blackeye goby, *Coryphopterus nicholsi* (Teleostei: Gobidae). Gen. Comp. Endocrinol. 118, 273–283.

Krotzer, M.J., 1990. The effects of induced masculinization on reproductive and aggressive behaviors of the female mosquitofish *Gambusia affinis affinis*. Environ. Biol. Fishes 29, 127–134.

Krueger, W.H., Oliveira, K., 1999. Evidence for environmental sex determination in the American eel, *Anguilla rostrata*. Environ. Biol. Fishes 55, 381–389.

Krug, H.M., 1990. The Azorean blackspot seabream *Pagellus bogaraveo* Brunnich 1768 Teleostei Sparidae reproductive cycle hermaphroditism maturity and fecundity. Cybium 14, 151–159.

Kruzynski, G.M., Birtwell, I.K., Rogers, I.H., 1984. Studies on chinook salmon (*Oncorhynchus tshawytscha*) and municipal waste from the Iona Island sewage treatment plant, Vancouver. Abstracts of papers presented at. 11th Annual Aquatic Toxicity Workshop, Richmond, B.C., Canada.

Kubota, Z., Hatakeyama, H., 1987. Influence of initial age and duration of estrone treatment on sex reversal of the loach *Misgurnus anguillicaudatus*. J. Shimonoseki Univ. Fish. 36, 29–38.

Kubota, Z., Nakajima, I., Watanabe, N., 1988. Influence of oral treatment of estrone on the sex reversal of the loach *Misgurnus anguillicaudatus*. J. Shimonoseki Univ. Fish. 36, 2–3.

Kuo, C.-M., Ting, Y.-Y., Yeh, S.-L., 1987. Induced sex reversal and spawning of blue-spotted grouper, *Epinephelus fario.* Induced Spawning Of Asian Fishes: Proceedings of a Fish Breeding Workshop, Singapore. Aquaculture 74, (1–2).

Kusen, J.D., Nakazono, A., 1991. Protogynous hermaphroditism in the parrotfish, *Calotomus japonicus*. Jpn. J. Ichthyol. 38, 41–46.

Kusen, J.D., Nakagawa, K., Yogo, Y., Nakazono, A., 1991. Protogynous hermaphroditism in the sand diver *Trichonotus filamentosus*. Bull. Jpn. Soc. Sci. Fish. 57, 35–40.

Kuwamura, T., 1984. Social structure of the protogynous fish *Labroides dimidiatus*. Publ. Seto Mar. Biol. Lab. 29, 1–3.

Kuwamura, T., Nakashima, Y., Yogo, Y., 1994. Sex change in either direction by growth-rate advantage in the monogamous coral goby, *Paragobiodon echinocephalus*. Behav. Ecol. 5, 434–438.

Kuwaye, T.T., Okimoto, D.K., Shimoda, S.K., Howerton, R.D., Lin, H.R., Pang, P.K.T., Grau, E.G., 1993. Effect of 17-alpha methyltestosterone on the growth of the euryhaline tilapia *Oreochromis mossambicus* in fresh water and in sea water. Aquaculture 113, 137–152.

Kwon, H.C., 1997. Effects of estradiol and pituitary hormones on in vitro vitellogenin synthesis in the eel, *Anguilla japonica*. J. Korean Fish. Soc. 30, 282–290.

Kwon, H.C., Hara, A., Mugiya, Y., Yamada, J., 1990. Enzyme linked-immunosorbent assay ELISA of vitellogenin in whitespotted charr *Salvelinus leucomaenis*. Bull. Fac. Fish., Hokkaido Univ. 41, 162–180.

Kwon, H.C., Hayashi, S., Mugiya, Y., 1993. Vitellogenin induction by estradiol-17 beta in primary hepatocyte culture in the rainbow trout, *Oncorhynchus mykiss*. Comp. Biochem. Physiol., B 104B, 381–386.

Kwon, J.Y., McAndrew, B.J., Penman, D.J., 2000. Inhibition of Aromatase Activity Suppresses High-Temperature Feminisation of Genetic Male Nile Tilapia, *Oreochromis niloticus* University of Bergen, Bergen, Norway.

Lagomarsino, I.V., Conover, D.O., 1993. Variation in environmental and genotypic sex-determining mechanisms across a latitudinal gradient in the fish, *Menidia menidia*. Evolution 47, 487–494.

Lahav, E., 1993. Use of sex-reversed females to produce all-male tilapia (*Oreochromis aureus*) fry. Isr. J. Aquacult. 45, 131–136.

Lahav, M., Lahav, E., 1990. The development of all-male tilapia hybrids in Nir David Israel. Isr. J. Aquacult.-Bamidgeh 42, 58–61.

Laidley, C.W., Thomas, P., 1997. Changes in plasma sex steroid binding protein levels associated with ovarian recrudescence in the spotted seatrout (*Cynoscion nebulosus*). Biol. Reprod. 56, 931–937.

Lakra, W.S., 1996. Cytogenetic studies on endangered fish species: 1. Karyotypes of three species of mahseers, *Tor putitora*, *T. tor* and *T. khudree* (Cyprinidae: Pisces). Cytobios 85, 205–218.

Lakra, W.S., John, G., Barat, A., 1997. Cytogenetic studies on endangered and threatened fishes II karyotypes of two species of snow-trout, *Schizothorax richardsonii* (Gray) and *S. kumaonensis* (Menon). Proc. Acad. Sci. India B 67, 79–81.

Laming, P.R., Ebbesson, S.O.E., 1984. Arousal and fright responses and their habituation in the slippery dick, *Halichoeres bivittatus*. Experientia 40, 767–768.

Lamrini, A., 1986. Sexuality of *Pagellus acarne* (Risso, 1826) (Teleostei Sparidae) from the southern Atlantic coast of Morocco (21 degree–26 degree N). Cybium 10, 3–14.

Lazier, C.B., Lonergan, K., Mommsen, T.P., 1985. Hepatic estrogen receptors and plasma estrogen-binding activity in the Atlantic salmon. Gen. Comp. Endocrinol. 57, 234–245.

Lazier, C.B., Langley, S., Ramsey, N.B., Wright, J.M., 1996. Androgen inhibition of vitellogenin gene expression in tilapia (*Oreochromis niloticus*). Gen. Comp. Endocrinol. 104, 321–329.

Le Bail, P.Y., Breton, B., 1981. Rapid determination of the sex of puberal salmonid fish by a technique of immunoagglutination. Aquaculture 22, 367–375.

Le Gac, F., Loir, M., Le Bail, P.Y., Ollitrault, M., 1996. Insulin-like growth factor (IGF-I) mRNA and IGF-I receptor in trout testis and in isolated spermatogenic and Sertoli cells. Mol. Reprod. Dev. 44, 23–35.

Le Guellec, K., Lawless, K., Valotaire, Y., Kress, M., Tenniswood, M., 1988. Vitellogenin gene expression in male rainbow trout (*Salmo gairdneri*). Gen. Comp. Endocrinol. 71, 359–371.

Leatherland, J.F., Sonstegard, R.A., 1980. Effect of dietary Mirex and PCBs in combination with food deprivation and testosterone administration on thyroid activity and bioaccumulation of organochlorines in rainbow trout *Salmo gairdneri* Richardson. J. Fish Dis. 3, 115–124.

Lebail, P.Y., 1981. Sex identification in relation to maturity stage in fish. Lab. Physiol. Poiss. 82, 1–82.

Lebrun, C.R., Billard, R., Jalabert, B., 1982. Changes in the number of germ cells in the gonads of the rainbow trout (*Salmo gairdneri*) during the first ten post-hatching weeks. Reprod., Nutr., Dev. 22, 405–412.

Lech, J.J., Lewis, S.K., Ren, L., 1996. In vivo estrogenic activity of nonylphenol in rainbow trout. Fundam. Appl. Toxicol. 30, 229–232.

Lee, Y.D., 1995. Sexual phenomenon of protogynous serranid fish. Exploit. Mar. Resour., 143–150.

Lee, J.S., Lee, Y.D., 1996. Early gonadogenesis and sex differentiation in the viviparous teleost, *Ditrema temmincki*. Bull. Korean Fish. Soc. 29, 34–43.

Lee, G.M., Wright Jr., J.E., 1981. Mitotic and Meiotic Analyses of Brook Trout, *Salvelinus fontinalis*. J. Hered. 72, 321–327.

Lee, Y.D., Rho, H.K., Lee, T.Y., 1991. Reproductive ecology of the wrasse, *Halicoeres poecilopterus* (Temmink et Schlegel). Bull. Mar. Res. Inst. Cheju Natl. Univ. 15, 93–102.

Lee, Y.D., Go, Y.B., Chung, S.C., 1992a. Reproductive cycle and sex reversal of the cock-tail wrasse, *Pteragogus flagellifera*. Bull. Mar. Res. Inst. Cheju Natl. Univ. 16, 43–53.

Lee, Y.D., An, C.M., Lee, J.J., Lee, T.Y., 1992b. Reproductive cycle and sex reversal of *Pseudolabrus japonicus* (Houttuyn). Bull. Mar. Res. Inst. Cheju Natl. Univ. 16, 55–66.

Lee, S.T.L., Kime, D.E., Chao, T.M., Lim, H.S., Chou, R., Lam, T.J., Tan, C.H., 1995. In vitro metabolism of testosterone by gonads of the grouper (*Epinephelus tauvina*) before and after sex inversion with 17-alpha-methyltestosterone. Gen. Comp. Endocrinol. 99, 41–49.

Lee, Y.D., Rho, S., Chang, Y.J., Baek, H.J., An, C.M., 1996. Sex differentiation of the rockfish, *Sebastes schlegeli*. J. Korean Fish. Soc. 29, 44–50.

Lee, C.H., Na, O.S., Yeo, I.K., Baek, H.J., Lee, Y.D., 2000. Effects of sex steroid hormones and high temperature on sex differentiation in black rockfish, *Sebastes schlegeli*. Bull. Korean Fish. Soc. 33, 373–377.

Leem, J.B., Sakamoto, K., Tsuruda, Y., Nakazono, A., 1998. Sexual pattern of the labrid fishes collected from Kuchinoerabu-jima, Kagoshima, Japan. J. Fac. Agric., Kyushu Univ. 42, 409–419.

Legouis, R., Cohens-Salmon, M., del Castillo, I., Petit, C., 1994. Isolation and characterization of the gene responsible for the X chromosome linked Kallman syndrome. Biomed. Pharmacother. 48, 241–246.
LeGrande, W.H., 1975. Karyology of six species of Louisiana flatfishes (Pleuronectiformes: Osteichthyes). Copeia 1975, 516–522.
LeGrande, W.H., 1981. Chromosomal evolution in North American catfishes (Siluriformes: Ictaluridae) with particular emphasis on the madtoms, *Noturus*. Copeia 1981, 33–52.
Lejeune, P., 1987. The effect of local stock density on social behavior and sex change in the Mediterranean labrid *Coris julis*. Environ. Biol. Fishes 18, 135–141.
Leonard, J.L., 1993. Sexual conflict in simultaneous hermaphrodites evidence from serranid fishes. Environ. Biol. Fishes 36, 135–148.
Lester, L.J., Lawson, K.S., Abella, T.A., Palada, M.S., 1989. Estimated heritability of sex ratio and sexual dimorphism in tilapia. Aquacult. Fish. Manage. 20, 369–380.
Levavi-Sivan, B., Yaron, Z., 1993. Intracellular mediation of GnRH action on GTH release in tilapia. Fish Physiol. Biochem. 11, 51–59.
Levavi-Sivan, B., Ofir, M., Yaron, Z., 1995. Possible sites of dopaminergic inhibition of gonadotropin release from the pituitary of a teleost fish, tilapia. Mol. Cell. Endocrinol. 109, 87–95.
Levavi-Zermonsky, B., Yaron, Z., 1986. Changes in gonadotropin and ovarian steroids associated with oocyte maturation during spawning induction in the carp. Gen. Comp. Endocrinol. 62, 89–98.
Levin, C.B., Foster, N.R., 1972. Cytotaxonomic studies in Cyprinodontinae: multiple sex chromosomes in *Germanella pulchra*. Not. Nat. 446, 1–5.
Li, Y., Gold, J.R., 1991. Cytogenetic studies in North American minnows (Cyprinidae): 22. chromosomal nucleolar organizer regions in the genus *Pimephales*. Can. J. Zool. 69, 2826–2830.
Lie, O., Slettan, A., Lingaas, F., Olsaker, I., Hordvik, I., Refstie, T., 1994. Haploid gynogenesis: a powerful strategy for linkage analysis in fish. Anim. Biotechnol. 5, 33–45.
Lieder, U., 1963. On presumptive sex chromosomes in Perca, Acerina and Anguilla. Biol. Zentalbl. 82, 296–302.
Lim, B.H., Phang, V.P.E., Reddy, P.K., 1992. The effects of short-term treatment of 17 alpha-methyltestosterone and 17 beta-estradiol on growth and sex ratio in the red variety of swordtail, *Xiphophorus helleri*. J. Aquacult. Trop. 7, 267–274.
Lin, Y., 1985. An approach to the factor causing the fertility of the hybrids of blunted snout bream (*Megalobrama amplycephala*) (female) × Chinese bream (*Parabramis pekinensis*) (male). J. Fish. China 9, 63–69.
Lin, H.R., Peter, R.E., 1996. Hormones and spawning in fish. Asian Fish. Sci. 9, 21–33.
Lin, D., Black, S.M., Nagahama, Y., Miller, W.L., 1993. Steroid 17-alpha hydroxylase and 17, 20 lyase activities of p450c17 contributions of serine-106 and p450 reductase. Endocrinology 132, 2498–2506.
Lin, F., Dabrowski, K., Timmermans, L.P.M., 1997. Early gonadal development and sexual differentiation in muskellunge (*Esox masquinongy*). Can. J. Zool. 75, 1262–1269.
Lindsey, C.C., 1962. Experimental study of meristic variation in a population of threespine stickleback, *Gasterosteus aculeatus*. Can. J. Zool., 40.
Linhart, O., Kvasnicka, P., Slechtova, V., Pokorny, J., 1986. Induced gynogenesis by retention of the second polar body in the common carp, *Cyprinus carpio* L., and heterozygosity of gynogenetic progeny in transferrin and Ldh-B super(1) loci. Aquaculture 54, 1–2.
Linhart, O., Slechtova, V., Kvasnicka, P., Rab, P., Prikryl, I., 1989. Chromosome manipulations in tench (*Tinca tinca* L.) and carp (*Cyprinus carpio* L.) in Czechoslovakia. Pr. VURH Vodnany 18, 53–60.
Linhart, O., Kvasnicka, P., Flajshans, M., Kasal, A., Rab, P., Palecek, J., Slechta, V., Hamackova, J., Prokes, M., 1995. Genetic studies with tench, *Tinca tinca* L.: induced meiotic gynogenesis and sex reversal. Aquaculture 132, 3–4.
Lisitsyn, N., Lisitsyn, N., Wigler, M., 1993. Cloning the difference between two genomes. Science 259, 946–951.
Liu, C.H., 1979. Study on the reproduction cycle and sex reversal of *Therapon jarbua* (Forskal). China Fish. Mon. 314, 10–14.
Liu, L., 1983. Studies on the chromosome G-banding patterns in rice-field eels (*Monopterus albus* Zuiew). Acta Genet. Sin. 10, 230–234.
Liu, J., Tian, M., 1991. A chromosome study on two sparid fishes (*Pagrosomus major* and *Sparus macrocephalus*). Mar. Sci. 3, 64–67.

Liu, S., Yao, Z., 1995. Self-fertilization of hermaphrodites of the teleost *Clarias lazerea* after oral administration of 17-alpha-methyltestosterone and their offspring. J. Exp. Zool. 273, 527–532.

Liu, Q., Goudie, C.A., Simco, B.A., Davis, K.B., 1993. Gene mapping of the sex-linked enzyme glucosephosphate isomerase-B in channel catfish: from discovery to commercialization. Eur. Aquacult. Soc. 19, 192.

Liu, Q., Goudie, C.A., Simco, B.A., Davis, K.B., 1996a. Sex-linkage of glucose phosphate isomerase-B and mapping of the sex-determining gene in channel catfish. Cytogenet. Cell Genet. 73, 282–285.

Liu, S., Yao, Z., Wang, Y., 1996b. Sex hormone induction of sex reversal in the teleost *Clarias lazera* and evidence for female homogamety and male heterogamety. J. Exp. Zool. 276, 432–438.

Liu, D., Le Drean, Y., Ekker, M., Xiong, F., Hew, C., 1997. Teleost FTZ-F1 homolog and its splicing variant determine the expression of the salmon gonadotropin II beta subunit gene. Mol. Endocrinol. 11, 877–890.

Liu, J., You, F., Wang, X., Xu, Y., Zhang, P., 1999. Chromosome and karyotype evidence of artificial-induced gynogenesis in the olive flounder *Paralichthys olivaceus*. Oceanol. Limnol. Sin. 30, 68–72.

Liu, S., Govoroun, M., D'Cotta, H., Ricordel, M.-J., Lareyre, J.-J., McMeel, O.M., Smith, T., Nagahama, Y., Guiguen, Y., 2000. Expression of cytochrome *P*45011beta (11beta-hydroxylase) gene during gonadal sex differentiation and spermatogenesis in rainbow trout, *Oncorhynchus mykiss*. J. Steroid Biochem. 75, 291–298.

Lloyd, M.A., Fields, M.J., Thorgaard, G.H., 1989. Bkm minisatellite sequences are not sex associated but reveal DNA fingerprint polymorphisms in rainbow trout. Genome 32, 865–868.

Lo Nostro, F.L., A, G.G., 1996. Presence of primary and secondary males in a population of the protogynous *Synbranchus marmoratus*. J. Fish Biol. 49, 788–800.

Lodi, E., 1978. Chromosome complement of the guppy, *Poecilia reticulata* Peters (Pisces, Osteichtyhyes). Caryologia 31, 475–477.

Lodi, E., 1979. Instances of sex inversion in the domesticated swordtail, *Xiphophorus helleri* Heckel (Pisces, Osteichthyes). Experientia 35, 1440–1441.

Lodi, E., 1980. Hermaphroditic and gonochoric populations of *Cobitis taenia bilineata* Canestrini (Cobitidae Osteichthyes). Monit. Zool. Ital. 14, 235–243.

Lodi, E., Marchionni, V., 1980. Chromosome complement of the masked loach *Sabanejewia larvata* (De Fil) (Pisces, Osteichthyes). Caryologia 33, 435–440.

Lokman, P.M., Young, G., 1998. An intersexual migratory (silver) longfinned New Zealand eel and its gonadal response to treatment with salmon pituitary homogenate. J. Fish Biol. 52, 547–555.

Lone, K.P., Ridha, M.T., 1993. Sex reversal and growth of *Oreochromis spilurus* guenther in brackish and sea water by feeding 17-alpha methyltestosterone. Aquacult. Fish. Manage. 24, 593–602.

Lopez, P.A., Fenocchio, A.S., 1994. Confirmation of two different cytotypes for the neotropical fish *Hoplias malabaricus* Gill 1903 (Charactiformes). Cytobios 80, 217–221.

Lou, Y., Song, T., Wang, Y., Wei, H., Wu, M., Xu, H., Wang, Z., 1994. Studies on sex control in allogynogenetic crucian carp. J. Fish. China 18, 169–176.

Lowartz, S.M., Beamish, F.W., 2000. Novel perspectives in sexual lability through gonadal biopsy in larval sea lampreys. J. Fish Biol. 56, 743–757.

Lowe, T.P., Larkin, J.R., 1975. Sex reversal in *Betta splendens* Regan with emphasis on the problem of sex determination. J. Exp. Zool. 191, 25–32.

Lubinski, B.A., Davis, W.P., Taylor, D.S., Turner, B.J., 1995. Outcrossing in a natural population of a self-fertilizing hermaphroditic fish. J. Hered. 86, 469–473.

Lucchesi, J.C., 1978. Gene dosage compensation and the evolution of sex chromosomes. Science 202, 711–716.

Luo, J.L., Wang, Z.X., Lin, Z.P., Yang, J.H., 1986. Studies on the karyotype of *Clarias fuscus*. J. Fish. China 10, 441–446.

Lutnesky, M., 1988. Sexual dimorphism, dichromatism, and protogynous hermaphroditism in the pomacanthid angelfish, *Centropyge potteri*. Pac. Sci. 42, 126.

Lutnesky, M., 1989. Stimulation, inhibition, and induction of "early" sex change in the pomacanthid angelfish *Centropyge potteri*. Pac. Sci. 43, 196–197.

Lutnesky, M.M.F., 1994. Density-dependent protogynous sex change in territorial-haremic fishes: models and evidence. Behav. Ecol. 5, 375–383.

Lutnesky, M.M.F., 1996. Size-dependent rate of protogynous sex change in the pomacanthid angelfish, *Centropyge potteri*. Copeia 1996, 209–212.

Lye, C.M., Frid, C.L.J., Gill, M.E., McCormick, D., 1997. Abnormalities in the reproductive health of flounder *Platichthys flesus* exposed to effluent from a sewage treatment works. Mar. Pollut. Bull. 34, 34–41.

Macchi, G.J., Christiansen, H., 1994. A case of hermaphroditism in white croaker, *Micropogonias furnieri*. Atlantica (Rio Grande) 16, 209–212.

MacKay, M.E., Raelson, J., Lazier, C.B., 1996. Up-regulation of estrogen receptor mRNA and estrogen receptor activity by estradiol in liver of rainbow trout and other teleostean fish. Comp. Biochem. Physiol., C 115C, 201–209.

Mackie, M., 2000. Reproductive biology of the halfmoon grouper, *Epinephelus rivulatus*, at Ningaloo Reef, Western Australia. Environ. Biol. Fishes 57, 363–376.

MacLatchy, D., L, P., J, N., G, V.D.K., 1997. Exposure to beta-sitosterol alters the endocrine status of goldfish differently than 17 beta-estradiol. Environ. Toxicol. Chem. 19, 1895–1904.

Maddock, M.B., Schwartz, F.J., 1996. Elasmobranch cytogenetics: methods and sex chromosomes. Bull. Mar. Sci. 58, 147–155.

Madigou, T., Le Goff, P., Salbert, G., Cravedi, J.P., Segner, H., Pakdel, F., Valotaire, Y., 2001. Effects of nonylphenol on estrogen receptor conformation, transcriptional activity and sexual reversion in rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 53, 173–186.

Madsen, S.S., Mathiesen, A.B., Korsgaard, B., 1997. Effects of 17 beta-estradiol and 4-nonylphenol on smoltification and vitellogenesis in Atlantic salmon (*Salmo salar*). Fish Physiol. Biochem. 17, 1–6.

Maestro, M., Planas, J., Gutierrez, J., Moriyama, S., Swanson, P., 1995. Effects of insulin-like growth factor I (IGF-I) on steroid production by isolated ovarian theca and granulosa layers of preovulatory coho salmon. Neth. J. Zool. 45, 143–146.

Mair, G.C., 1993. Chromosome-set manipulation in tilapia — techniques, problems and prospects. Genetics in aquaculture IV. Aquaculture 111, 227–244.

Mair, G.C., Penman, D.J., Scott, A.G., Skibinski, D.O.F., Beardmore, J.A., 1987. Hormonal sex-reversal and the mechanisms of sex determination in *Oreochromis*. Sel. Hybrid. Genet. Eng. Aquacult. 18, 18–19.

Mair, G.C., Beardmore, J.A., Skibinski, D.O.F., 1989. Experimental evidence for environmental sex determination in *Oreochromis* species. Proceedings of the Second Asian Fisheries Forum, Tokyo, Japan. The Second Asian Fisheries Forum, Tokyo, Japan, pp. 555–558.

Mair, G.C., Scott, A.G., Penman, D.J., Skibinski, D.O.F., Beardmore, J.A., 1991a. Sex determination in the genus *Oreochromis*: 2. Sex reversal, hybridisation, gynogenesis and triploidy in *O. aureus* Steindachner. Theor. Appl. Genet. 82, 153–160.

Mair, G.C., Scott, A.G., Penman, D.J., Beardmore, J.A., Skibinski, D.O.F., 1991b. Sex determination in the genus *Oreochromis*: I. Sex reversal, gynogenesis and triploidy in *O. niloticus* (L.). Theor. Appl. Genet. 82, 144–152.

Mair, G.C., Abucay, J.S., Beardmore, J.A., Skibinski, D.O.F., 1995. Growth performance trials of genetically male tilapia (GMT) derived from YY-males in *Oreochromis niloticus* L.: on station comparisons with mixed sex and sex reversed male populations. Aquaculture 137, 1–4.

Mair, G.C., Abucay, J.S., Skibinski, D.O.F., Abella, T.A., Beardmore, J.A., 1997. Genetic manipulation of sex ratio for the large-scale production of all-male tilapia, *Oreochromis niloticus*. Can. J. Fish. Aquat. Sci. 54, 396–404.

Maistro, E.L., Mata, E.P., Oliveira, C., Foresti, F., 1998. Unusual occurrence of a ZZ/ZW sex-chromosome system and supernumerary chromosomes in *Characidium* cf. fasciatum (Pisces, Characiformes, Characidiinae). Genetica 104, 1–7.

Maitre, J.L., Mercier, L., Dolo, L., Valotaire, Y., 1985. Characterization of specific estradiol receptors, induction of vitellogenin and vitellogenin mRNA in the liver of rainbow trout (*Salmo gairdnerii*). Biochimie 67, 215–225.

Majumdar, K.C., McAndrew, B.J., 1983. Sex ratios from interspecific crosses within the tilapias. In: Fishelson, L., Yaron, Z. (Eds.), International Symposium on Tilapia in Aquaculture. Tel Aviv University, Tel Aviv, pp. 261–269.

Majumdar, K.C., McAndrew, B.J., 1986. Relative DNA content of somatic nuclei and chromosomal studies in three genera, *Tilapia*, *Sarotherodon*, and *Oreochromis* of the tribe Tilapiini (Pisces, Cichlidae). Genetica 68, 175–188.

Malison, J.A., Garcia Abiado, M.A.R., 1996. Sex control and ploidy manipulations in yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum*). J. Appl. Ichthyol. 12, 189–194.

Malison, J.A., Kayes, T.B., Best, C.D., Amundson, C.H., Wentworth, B.C., 1986. Sexual differentiation and use of hormones to control sex in yellow perch (*Perca flavescens*). Can. J. Fish. Aquat. Sci. 43, 26–35.
Mandich, A.M.G.M.A., 1998. In vitro steroid secretion by ovarian follicles of the grouper *Epinephelus marginatus*. Trends in Comparative Endocrinology and Neurobiology: From Molecular to Integrative Biology, The New York Academy of Sciences, 15 May 1998. Ann. N. Y. Acad. Sci. 839, 586–588.
Mann, B.Q., Buxton, C.D., 1998. The reproductive biology of *Diplodus sargus* capensis and *Diplodus cervinus* hottentotus (Sparidae) off the South-East Cape coast, South Africa. Cybium 22, 31–47.
Manna, G.K., 1989. Fish cytogenetics related to taxonomy, evolution and monitoring aquatic genotoxic agents. In: Das, P., Jingran, A.G. (Eds.), Fish Genetics in India. Proceedings of the Symposium on Conservation and Management of Fish Genetic Resources of India. Today and Tomorrow's Printers and Publishers, New Delhi, India, pp. 21–46.
Manna, G.K., Khuda-Bukhsh, A.R., 1977. Karyomorphology of cyprinid fishes and cytological evaluation of the family. Nucleus 20, 119–127.
Manna, G.K., Prasad, R., 1977. Chromosome analysis in five species of fresh water fishes. Nucleus 20, 264–271.
Manning, N.J., Kime, D.E., 1984. Temperature regulation of ovarian steroid production in the common carp, *Cyprinus carpio* L., in vivo and in vitro. Gen. Comp. Endocrinol. 56, 376–388.
Manning, N.J., Kime, D.E., 1985. The effect of temperature on testicular steroid production in the rainbow trout, *Salmo gairdneri*, in vitro and in vivo. Gen. Comp. Endocrinol. 57, 377–382.
Manzoor Ali, P.K.M., Satyanarayana Rao, G.P., 1989. Growth improvement in carp, *Cyprinus carpio* (Linnaeus), sterilized with 17 alpha-methyltestosterone. Aquaculture 76, 1–2.
Mao, L., Jin, X., 1994. Analysis of chromosome karyotype of *Enedrias nebulosus*. J. Dalian Fish. Coll. 9, 32–35.
Mao, L., Qiu, P., 1996. Karyotype analysis of *Alectrias benjamini*. J. Dalian Fish. Coll. 11, 37–42.
Mao, L., Yang, L., Qin, K., 1993. A comparative study on karyotypes of two species of gobioid fishes. J. Dalian Fish. Coll. 8, 1–7.
Marchand, O., Govoroun, M., D'Cotta, H., McMeel, O., Lareyre, J.-J., Bernot, A., Laudet, V., Guiguen, Y., 2000. DMRT1 expression during gonadal differentiation and spermatogenesis in the rainbow trout, *Oncorhynchus mykiss*. Biochim. Biophys. Acta 1493, 180–187.
Marengoni, N.G., Onoue, Y., 1998. Ultraviolet-induced androgenesis in Nile tilapia, *Oreochromis niloticus* (L.), and hybrid Nile × blue tilapia, *O. aureus* (Steindachner). Aquacult. Res. 29, 359–366.
Marian, T., Krasznai, Z., 1982. Karyological pattern of blue catfish (*Ictalurus furcatus* Ictaluridae). Aquacult. Hung. 3, 17–21.
Marin, I., Baker, B.S., 1998. The evolutionary dynamics of sex determination. Science 281, 1990–1994.
Martin-Robichaud, D.J., Peterson, R.H., Benfey, T.J., Crim, L.W., 1994. Direct feminization of lumpfish (*Cyclopterus lumpus* L.) using 17 beta-oestradiol-enriched *Artemia* as food. Aquaculture 123, 1–2.
Matsubara, T., Sawano, K., 1992. Sex determination of Pacific halibut *Hippoglossus stenolepis* Schmidt by the immunodot-blotting technique using antiserum against vitellogenin. Bull. Hokkaido Natl. Fish. Res. Inst. 56, 17–26.
Matsuda, M., Kusama, T., Oshiro, T., Kurihara, Y., Hamaguchi, S., Sakaizumi, M., 1997. Isolation of a sex chromosome-specific DNA sequence in the medaka, *Oryzias latipes*. Genes Genet. Syst. 72, 263–268.
Matsuda, M., Matsuda, C., Hamaguchi, S., Sakaizumi, M., 1998. Identification of the sex chromosomes of the medaka, *Oryzias latipes*, by fluorescence in situ hybridization. Cytogenet. Cell Genet. 82, 257–262.
Matsuda, M., Sotoyama, S., Hamaguchi, S., Sakaizumi, M., 1999. Male-specific restriction of recombination frequency in the sex chromosomes of the medaka, *Oryzias latipes*. Genet. Res. 73, 225–231.
Matsuda, M., Nagahama, Y., Shinomiya, A., Sato, T., Matsuda, C., Kobayashi, T., Morrey, C.E., Shibata, N., Asakawa, S., Shimizu, N., Hori, H., Hamaguchi, S., Sakaizumi, M., 2002. A *Y*-specific, DM-domain gene, *DMY*, is required for male development in the medaka (*Oryzias latipes*). Nature (in press).
Matsuyama, M., Lara, R.T., Matsuura, S., 1988. Juvenile bisexuality in the red sea bream, *Pagrus major*. Environ. Biol. Fishes 21, 27–36.
Matta, M.B., Cairncross, C., Kocan, R.M., 1998. Possible effects of polychlorinated biphenyls on sex determination in rainbow trout. Environ. Toxicol. Chem. 17, 26–29.
Mauricio Nava-Bautista, J., Rodriguez-Gutierrez, M., 1997. Effect of 17 alpha-methyltestosterone and vitamin B complex on the sexual reversion induction of two of the development stages of *Xiphophorus helleri*, Heckel, 1848 (Pisces: Poecilidae). J. Aquacult. Trop. 12, 65–71.

May, B., Grewe, P.M., 1993. Fate of maternal MtDNA following cobalt-60 inactivation of maternal nuclear DNA in unfertilized salmonid eggs. Genome 36, 725–730.

May, B., Johnson, K.R., Wright Jr., J.E. 1989. Sex linkage in salmonids: evidence from a hybridized genome of Brook trout and Arctic charr. Biochem. Genet. 27, 291–302.

Mayer, I., Berglund, I., Rydevik, M., Borg, B., Schulz, R., 1990. Plasma levels of five androgens and 17 alpha-hydroxy-20 beta-dihydroprogesterone in immature and mature male Baltic salmon (*Salmo salar*) parr, and the effects of castration and androgen replacement in mature parr. Can. J. Zool. 68, 263–267.

Mayer, I., Schmitz, M., Borg, B., Schulz, R., 1992. Seasonal endocrine changes in male and female arctic charr *Salvelinus alpinus* I. Plasma levels of three androgens 17-alpha hydroxy-20-beta-dihydroprogesterone and 17-beta estradiol. Can. J. Zool. 70, 37–42.

McConnell, S.K.J., Skibinski, D.O.F., Beardmore, J.A., 1996. A search for sex-specific DNA regions in *Oreochromis niloticus*. ICLARM Conference Proceedings, Makati City, Philippines. ICLARM, Makati City, Philippines, pp. 349–353.

McElreavey, K., Vilain, E., Herskowitz, I., Fellous, M., 1993. A regulatory cascade hypothesis for mammalian sex determination: SRY represses an negative regulator of male development. Proc. Natl. Acad. Sci. U. S. A. 90, 3368–3372.

McKay, S.J., Devlin, R.H., Smith, M.J.A., 1996. Phylogeny of Pacific salmon and trout based on growth hormone type-2 and mitochondrial NADH dehydrogenase subunit 3 DNA sequences. Can. J. Fish. Aquat. Sci. 53, 1165–1176.

McMaster, M.E., Kraak, G.d., Portt, C.B., Munkittrick, K.R., Sibley, P.K., Smith, I.R., Dixon, D.G., 1991. Changes in hepatic mixed-function oxygenase (MFO) activity, plasma steroid levels and age at maturity of a white sucker (*Catostomus commersoni*) population exposed to bleached kraft pulp mill effluent. Aquat. Toxicol. 21, 199–218.

McMaster, M.E., Van der Kraak, G.J., Munkittrick, K.R., 1995. Exposure to bleached kraft pulp mill effluent reduces the steroid biosynthetic capacity of white sucker ovarian follicles. Comp. Biochem. Physiol., C 112C, 169–178.

McPherson, G.R., 1977. Sex change in the wrasse *Pseudolabrus gymnogenis* (Labridae). Aust. Zool. 19, 185–200.

Mei, W., Cao, Y., Li, R., Shi, B., Hu, A., 1993. A preliminary observation on sex reversal of the mud eel *Monopterus albus*. J. Zhenjiang Coll. Fish./Zhenjiang Shuichan Xueyuan Xuebao 12, 53–58.

Melamed, P., Gur, G., Elizur, A., Rosenfeld, H., Sivan, B., Rentier-Delrue, F., Yaron, Z., 1996. Differential effects of gonadotropin-releasing hormone, dopamine and somatostatin and their second messengers on the RNA levels of gonadotropin II beta subunit and growth hormone in the teleost fish, tilapia. Neuroendocrinology 64, 320–328.

Melamed, P., Gur, G., Rosenfeld, H., Elizur, A., Yaron, Z., 1997. The mRNA levels of GtH I beta, GtH II beta and GH in relation to testicular development and testosterone treatment in pituitary cells of male tilapia. Fish Physiol. Biochem. 17, 93–98.

Melamed, P., Rosenfeld, H., Elizur, A., Yaron, Z., 1998. Endocrine regulation of gonadotropin and growth hormone gene transcription in fish. Comp. Biochem. Physiol., C 119C, 325–338.

Melamed, P., Gur, G., Rosenfeld, H., Elizur, A., Yaron, Z., 1999. Possible interactions between gonadotrophs and somatotrophs in the pituitary of tilapia: apparent roles for insulin-like growth factor I and estradiol. Endocrinology 140, 1183–1191.

Melamed, P., Gur, G., Rosenfeld, H., Elizur, A., Schulz, R., Yaron, Z., 2000. Reproductive development of male and female tilapia hybrids (*Oreochromis niloticus* × *O. aureus*) and changes in mRNA levels of gonadotropin (GtH) I beta and II beta subunits. J. Exp. Zool. 286, 64–75.

Melard, C., 1995. Production of a high percentage of male offspring with 17 alpha-ethynylestradiol sex-reversed *Oreochromis aureus*: 1. Estrogen sex-reversal and production of F2 pseudofemales. Aquaculture 130, 25–34.

Melard, C., Desprez, D., Philippart, J.C., 1994. Sex control in tilapia: overview and perspectives of 10 years experiences at the aquaculture research station of Tihange. Cah. Ethol. Fond. Appl., Anim. Hum. 13, 421–434.

Mellanen, P., Petaenen, T., Lehtimaeki, J., Maekelae, S., Bylund, G., Holmbom, B., Mannila, E., Oikari, A., Santti, R., 1996. Wood-derived estrogens: studies in vitro with breast cancer cell lines and in vivo in trout. Toxicol. Appl. Pharmacol. 136, 381–388.

Meriwether, F.H., Torrans, E.L., 1986. Evaluation of a new androgen (mibolerone) and procedure to induce functional sex reversal in tilapia. Proceedings of the First Asian Fisheries Forum, Manila, Phillipines. The First Asian Fisheries Forum, Manila, Phillipines, pp. 675–678.

Mestriner, C.A., Bertollo, L.A., Galetti Junior, P.M., 1995. Chromosome banding and synaptonemal complexes in *Leporinus lacustris* (Pisces, Anostomidae): analysis of a sex system. Chromosome Res. 3, 440–443.

Meyer, K., 1977. Reproductive behavior and patterns of sexuality in the Japanese labrid fish *Thalassoma cupido*. Jpn. J. Ichthyol. 24, 101–112.

Mezhnin, F.I., 1978. Development of the sex cells in the early ontogeny of the common perch, *Perca fluviatilis*. J. Ichthyol. 18, 71–86.

Micale, V., Perdichizzi, F., 1994. Further studies on the sexuality of the hermaphroditic teleost *Diplodus sargus*, with particular reference to protandrous sex inversion. J. Fish Biol. 45, 661–670.

Michele, J.L., Takahashi, C.S., 1977. Comparative cytology of *Tilapia rendalli* and *Geophagus brasilliensis* (Cichlidae, Pisces). Cytologia 42, 535–537.

Michele, J.L., Takahashi, C.S., Ferrari, I., 1977. Karyotypic study of some species of the family Loricariidae (Pisces). Cytologia 42, 539–546.

Middaugh, D.P., Hemmer, M.J., 1987. Influence of environmental temperature on sex-ratios in the tidewater silverside, *Menidia peninsulae* (Pisces: Atherinidae). Copeia 1987, 958–964.

Mikolajczyk, T., Weil, C., Breton, B., 1993. Nicotine stimulates maturational gonadotropin GtH-2 release from carp *Cyprinus carpio* L. pituitary cells. Comp. Biochem. Physiol., C 105, 83–88.

Mikolajczyk, T., Chyb, J., Sokolowska-Mikolajczyk, M., Breton, B., Monden, K., Epler, P., 1998. Nicotine stimulated GtH2 secretion in vivo in male common carp (*Cyprinus carpio*); potentiation of GnRH action and possible interaction with dopaminergic system. Aquat. Living Resour. 11, 155–161.

Miller, R.R., Uyeno, T., 1980. *Allodontichthys hubbsi*, a new species of goodeid fish from south-western Mexico. Occas. Pap. Mus. Zool. Univ. Mich. 692, 1–13.

Milton, D.A., Chenery, S.R., Farmer, M.J., Blaber, S.J.M., 1997. Identifying the spawning estuaries of the tropical shad, terubok *Tenualosa toli*, using otolith microchemistry. Mar. Ecol.: Prog. Ser. 153, 283–291.

Mims, S.D., Shelton, W.L., Linhart, O., Wang, C., 1997. Induced meiotic gynogenesis of paddlefish *Polyodon spathula*. J. World Aquacult. Soc. 28, 334–343.

Miranda, L.A., Strussmann, C.A., Somoza, G.M., 2001. Immunocytochemical identification of GtH1 and GtH2 cells during the temperature-sensitive period for sex determination in pejerrey, *Odontesthes bonariensis*. Gen. Comp. Endocrinol. 124, 45–52.

Mirza, J.A., Shelton, W.L., 1988. Induction of gynogenesis and sex reversal in silver carp. Aquaculture 68, 1–14.

Miura, T., Yamauchi, K., Takahashi, H., Nagahama, Y., 1991. Hormonal induction in vitro of all stages of spermatogenesis in the male Japanes eel (*Anguilla japonica*). Proc. Natl. Acad. Sci. U. S. A. 88, 5774–5778.

Miura, K., Kusakari, M., Takano, K., 1994. Distinction of the sex of Japanese flounder, *Paralichthys olivaceus*, using immunodiffusion test. Sci. Rep. Hokkaido Fish. Exp. Stn. 44, 19–23.

Miura, C., Miura, T., Yamashita, M., Yamauchi, K., Nagahama, Y., 1996. Hormonal induction of all stages of spermatogenesis in germ-somatic cell coculture from immature Japanese eel testis. Dev., Growth Differ. 38, 257–262.

Miura, T., Kudo, N., Miura, C., Yamauchi, K., Nagahama, Y., 1998. Two testicular cDNA clones suppressed by gonadotropin stimulation exhibit ZP2- and ZP3-like structures in Japanese eel. Mol. Reprod. Dev. 51, 235–242.

Miwa, S., Yan, L., Swanson, P., 1994. Localization of two gonadotropin receptors in the salmon gonad by in vitro ligand autoradiography. Biol. Reprod. 50, 629–642.

Miya, M., Nemoto, T., 1985. Protandrous sex reversal in *Cyclothone atraria* (Family Gonostomatidae). Jpn. J. Ichthyol. 31, 438–439.

Moiseyeva, Y., 1983. The development of the gonads of the round goby, *Neogobius melanostomus* (Gobiidae), during the embryonic period. J. Ichthyol. 23, 64–74.

Mommsen, T.P., Lazier, C.B., 1986. Stimulation of estrogen receptor accumulation by estradiol in primary cultures of salmon hepatocytes. FEBS Lett. 195, 269–271.

Monaco, P.J., Rasch, E.M., Balsano, J.S., 1984. Apomictic reproduction in the Amazon molly, *Poecilia formosa* and its triploid hybrids. Evol. Genet., 311–327.

Montero, M., Lebelle, N., King, J.A., Millar, R.P., Dufour, S., 1995. Differential regulation of the two forms of gonadotropin-releasing hormone (mGnRH and cGnRH-II) by sex steroids in the European female silver eel (*Anguilla anguilla*). Neuroendocrinology 61, 525–535.

Moore, R., 1979. Natural sex inversion in the giant perch (*Lates calcarifer*). Aust. J. Mar. Freshwater Res. 30, 803–813.

Moore, C., Labisky, R.F., 1984. Population parameters of a relatively unexploited stock of snowy grouper in the lower Florida Keys. Trans. Am. Fish. Soc. 113, 322–329.

Morais da Silva, S., Hacker, A., Harley, V., Goodfellow, P., Swain, A., Lovell-Badge, R., 1996. Sox9 expression during gonadal development implies a conserved role for the gene in testis differentiation in mammals and birds. Nat. Genet. 14, 62–68.

Moran, P., Martinez, J.L., Garcia-Vazquez, E., Pendas, A.M., 1996. Sex chromosome linkage of 5S rDNA in rainbow trout (*Oncorhynchus mykiss*). Cytogenet. Cell Genet. 75, 145–150.

Moreira, O., Bertollo, L.A.C., Galetti Jr., P.M. 1985. Karyotypic study of some species of family Parodontidae (Pisces–Cypriniformes). Caryologia 38, 47–55.

Moreira-Filho, O., Bertollo, L.A.C., Galetti Jr., P.M., 1993. Distribution of sex chromosome mechanisms in neotropical fish and description of ZZ–ZW system in *Parodon hilarii* (Parodontidae). Caryologia 46, 115–125.

Morelli, S., Bertollo, L.A., Foresti, F., Moreira, O., de Almeida Toledo, F.S., 1983. Cytogenetic considerations on the genus *Astyanax* (Pisces, Characidae): I. Karyotypic variability. Caryologia 36, 235–244.

Morescalchi, A., Pisano, E., Stanyon, R., Morescalchi, M.A., 1992a. Cytotaxonomy of antarctic teleosts of the pagothenia and trematomus complex Nototheniidae Perciformes. Polar Biol. 12, 553–558.

Morescalchi, A., Hureau, J.C., Olmo, E., Ozouf Costaz, C., Pisano, E., Stanyon, R., 1992b. A multiple sex-chromosome system in Antarctic icefishes. Polar Biol. 11, 655–661.

Mori, T., Kawamata, K., Mizuno, S., Adachi, S., Yamauchi, K., 1995. The feminization of the barfin flounder, *Verasper moseri* by oral administration of estradiol-17 beta. Sci. Rep. Hokkaido Fish. Exp. Stn. 46, 1–6.

Mori, T., Matsumoto, H., Yokota, H., 1998. Androgen-induced vitellogenin gene expression in primary cultures of rainbow trout hepatocytes. J. Steroid Biochem. 67, 133–141.

Morita, S., Matsuyama, M., Kashiwagi, M., 1997. Seasonal changes of gonadal histology and serum steroid hormone levels in the bambooleaf wrasse *Pseudolabrus japonicus*. Bull. Jpn. Soc. Sci. Fish./Nippon Suisan Gakkaishi 63, 694–700.

Morizot, D.C., Slaugenhaupt, S.A., Kallman, K.D., Chakravarti, A., 1991. Genetic linkage map of fishes of the genus *Xiphophorus* (Teleostei: Poeciliidae). Genetics 127, 399–410.

Morrey, C.E., Nakamura, M., Kobayashi, T., Grau, E.G., Nagahama, Y., 1998. *P*450scc-like immunoreactivity throughout gonadal restructuring in the protogynous hermaphrodite *Thalassoma duperrey*. Int. J. Dev. Biol. 42, 811–816.

Morris, M.R., Batra, P., Ryan, M.J., 1992. Male–male competition and access to females in the swordtail *Xiphophorus nigrensis*. Copeia 1992, 980–986.

Moser, M., Whipple, J., Sakanari, J., Reilly, C., 1983. Protandrous hermaphroditism in striped bass from Coos Bay, Oregon. Trans. Am. Fish. Soc. 112, 567–569.

Mourot, B., Le Bail, P.Y., 1995. Enzyme-linked immunosorbent assay (ELISA) for rainbow trout (*Oncorhynchus mykiss*) vitellogenin. J. Immunoassay 16, 365–377.

Moyer, J., Nakazono, A., 1978a. Population structure, reproductive behavior and protogynous hermaphroditism in the angelfish *Centropyge interruptus* at Miyake-jima, Japan. Jpn. J. Ichthyol. 25, 25–39.

Moyer, J., Nakazono, A., 1978b. Protandrous hermaphroditism in six species of the anemonefish genus *Amphiprion* in Japan. Jpn. J. Ichthyol. 25, 101–106.

Moyer, J.T., Zaiser, M.J., 1984. Early sex change: a possible mating strategy of *Centropyge* angelfishes (Pisces: Pomacanthidae). J. Ethol. 2, 63–67.

Muller, U., Wolf, U., 1979. Cross-reactivity to mammalian anti-H–Y antiserum in teleostean fish. Differentiation 14, 185–187.

Müller-Belecke, A., Hörstgen-Schwark, G., 1995. Sex determination in tilapia (*Oreochromis niloticus*) sex ratios in homozygous gynogenetic progeny and their offspring. Aquaculture 137, 57–65.

Munday, P.L., Caley, M.J., Jones, G.P., 1998. Bi-directional sex change in a coral-dwelling goby. Behav. Ecol. Sociobiol. 43, 371–377.

Munkittrick, K.R., McMaster, M.E., Portt, C.B., Van Der Kraak, G.J., Smith, I.R., Dixon, D.G., 1992. Changes in maturity plasma sex steroid levels hepatic mixed-function oxygenase activity and the presence of external lesions in lake whitefish *Coregonus clupeaformis* exposed to bleached kraft mill effluent. Can. J. Fish. Aquat. Sci. 49, 1560–1569.

Murata, K., Sugiyama, H., Yasumasu, S., Iuchi, I., Yasumasu, I., Yamagami, K., 1997. Cloning of cDNA and estrogen-induced hepatic gene expression for choriogenin H, a precursor protein of the fish egg envelope (chorion). Proc. Natl. Acad. Sci. U. S. A. 94, 2050–2055.

Murofushi, M., 1980. Cytogenetical studies on fishes: III. Multiple sex chromosome mechanism in the filefish, *Stephanolepis cirrhifer*. Jpn. J. Genet. 35, 127–132.

Murofushi, M., Yosida, T.H., 1984. Cytogenetical studies on fishes: VIII. XX–Y sex chromosome mechanism newly found in the snake eel, *Muraenicthys gymnotus* (Anguilliform, Pisces). Proc. Jpn. Acad., Ser. B 60B, 21–23.

Murofushi, M., Nishikawa, S., Yosida, T.H., 1984. Kromosomo 34, 1079.

Murofushi, M., Nakatsubo, T., Smith, P.J., 1989. Karyological study on the new zealand leatherjacket *Parika scaber* fish of the order tetraodontiformes. Bull. Biogeogr. Soc. Jpn. 44, 35–38.

Myers, J.M., Penman, D.J., Basavaraju, Y., Powell, S.F., Baoprasertkul, P., Rana, K.J., Bromage, N., McAndrew, B.J., 1995. Induction of diploid androgenetic and mitotic gynogenetic Nile tilapia (*Oreochromis niloticus* L.). Theor. Appl. Genet. 90, 205–210.

Mylonas, C.C., Scott, A.P., Zohar, Y., 1997a. Plasma gonadotropin II, sex steroids, and thyroid hormones in wild striped bass (*Morone saxatilis*) during spermiation and final oocyte maturation. Gen. Comp. Endocrinol. 108, 223–236.

Mylonas, C.C., Scott, A.P., Vermeirssen, E.L., Zohar, Y., 1997b. Changes in plasma gonadotropin II and sex steroid hormones, and sperm production of striped bass after treatment with controlled-release gonadotropin-releasing hormone agonist-delivery systems. Biol. Reprod. 57, 669–675.

Mylonas, C.C., Magnus, Y., Klebanov, Y., Gissis, A., Zohar, Y., 1997c. Reproductive biology and endocrine regulation of final oocyte maturation of captive white bass. J. Fish Biol. 51, 234–250.

Nagahama, Y., 1987. 17 alpha, 20 beta-Dihydroxy-4-pregnen-3-one: a teleost maturation-inducing hormone. Dev., Growth Differ. 29, 1–12.

Nagahama, Y., 1994. Endocrine regulation of gametogenesis in fish. Int. J. Dev. Biol. 38, 217–229.

Nagahama, Y., 1997. 17-alpha, 20-beta-dihydroxy-4-pregnen-3-one, a maturation-inducing hormone in fish oocytes: mechanisms of synthesis and action. Steroids 62, 190–196.

Nagahama, Y., 1999. Gonadal steroid hormones: major regulators of gonadal sex differentiation and gametogenesis in fish. Sixth Int. Symp. on the Reproductive Physiology of Fish, Bergen, Norway.

Nagahama, Y., Yamashita, M., 1988. Mechanisms of synthesis and action of 17 alpha, 20 beta-dihydroxy-4-pregnen-3-one, a teleost maturation inducing substance. Fish Physiol. Biochem. 7, 193–200.

Nagahama, Y., Kagawa, H., Young, G., 1982. Cellular sources of sex steroids in teleost gonads. Can. J. Fish. Aquat. Sci. 39, 56–64.

Nagahama, Y., Hirose, K., Young, G., Adachi, S., Suzuki, K., Tamaoki, B.I., 1983. Relative in vitro effectiveness of 17 alpha, 20 beta-dihydroxy-4-pregnen-3-one and other pregnene derivatives on germinal vesicle breakdown in oocytes of ayu (*Plecoglossus altivelis*), amago salmon (*Oncorhynchus rhodurus*), rainbow trout (*Salmo gairdneri*), and goldfish (*Carassius auratus*). Gen. Comp. Endocrinol. 51, 15–25.

Nagahama, Y., Miura, T., Kobayashi, T., Ding, J., 1997. The Role of Activin in Spermatogenesis in Fish. Springer-Verlag, New York, pp. 196–203.

Nagaraj, C.G., Rao, G.P.S., 1987. Effect of testosterone acetate and estradiol benzoate on sexuality and growth of *Cyprinus carpio* (Linn.). The First Indian Fisheries Forum, Proceedings, Mangalore, India, 115–117.

Nagelkerken, W., 1979. Some aspects of transitionals of the grouper *Epinephelus cruentatus*. Proc. Assoc. Isl. Mar. Lab. Caribb. 14, 21.

Nagler, J.J., Bouma, J., Thorgaard, G.H., Dauble, D.D., 2001. High incidence of a male-specific genetic marker in phenotypic female chinook salmon from the Columbia River. Environ. Health Perspect. 109, 67–69.

Nagy, A., Rajki, K., Horvath, L., Csanyi, V., 1978. Investigation on carp *Cyprinus carpio* L. gynogenesis. J. Fish Biol. 13, 215–224.

Nagy, A., Bercsenyi, M., Csanyi, V., 1981. Sex reversal in carp (*Cyprinus carpio*) by oral administration of methyltestosterone. Can. J. Fish. Aquat. Sci. 38, 725–728.

Nagy, A., Csanyi, V., Bakos, J., Bercsenyi, M., 1984. Utilization of gynogenesis and sex-reversal in commercial carp breeding: growth of the first gynogenetic hybrids. Aquacult. Hung. 4, 7–16.

Nakamura, M., 1975. Dosage-dependent changes in the effect of oral administration of methyltestosterone on gonadal sex differentiation in *Tilapia mossambica*. Bull. Fac. Fish., Hokkaido Univ. 26, 99–108.

Nakamura, M., 1981. Effect of 11-ketotestosterone on gonadal sex differentiation in *Tilapia mossambica*. Bull. Fac. Fish., Hokkaido Univ. 47, 1323–1327.

Nakamura, M., 1982. Gonadal sex differentiation in whitespotted char, *Salvelinus leucomaenis*. Jpn. J. Ichthyol. 28, 431–436.

Nakamura, M., 1984. Effects of estradiol-17 beta on gonadal sex differentiation in two species of salmonids, the masu salmon, *Oncorhynchus masou*, and the chum salmon, *O. keta*. Aquaculture 43, 1–3.

Nakamura, M., 1994. A study of susceptibility of sex reversal after a single 2-hour treatment of androgen in amago salmon. Fish. Sci. Tokyo 60, 483–484.

Nakamura, M., Iwahashi, M., 1982. Studies on the practical masculinization in *Tilapia nilotica* by the oral administration of androgen. Bull. Jpn. Soc. Sci. Fish. 48, 763–769.

Nakamura, M., Nagahama, Y., 1985. Steroid producing cells during ovarian differentiation of the tilapia, *Sarotherodon niloticus*. Dev., Growth Differ. 27, 701–708.

Nakamura, M., Nagahama, Y., 1993. Ultrastructural study on the differentiation and development of steroid-producing cells during ovarian differentiation in the amago salmon *Oncorhynchus rhodurus*. Aquaculture 112, 237–251.

Nakamura, M., Takahashi, H., 1973. Gonadal sex differentiation in *Tilapia mossambica* with special regard to the time of estrogen treatment effective in inducing feminization of genetic fishes. Bull. Fac. Fish., Hokkaido Univ. 24, 1–13.

Nakamura, M., Takahashi, H., Hiroi, O., 1974. Sex differentiation of the gonad in the Masu salmon (*Oncorhynnchus masou*). Sci. Rep. Hokkaido Salmon Hatchery 28, 1–8.

Nakamura, D., Wachtel, S.S., Kallman, K., 1984. H–Y antigen and the evolution of heterogamety. J. Hered. 75, 353–358.

Nakamura, M., Nagahama, Y., Iwahashi, M., Kojima, M., 1987. Ovarian structure and plasma steroid hormones of triploid female rainbow trout. Bull. Jpn. Soc. Sci. Fish./Nippon Suisan Gakkaishi 53, 105.

Nakamura, M., Hourigan, T.F., Yamauchi, K., Nagahama, Y., Grau, E.F., 1989. Histological and ultrastructural evidence for the role of gonadal steroid hormones in sex change in the protogynous wrasse *Thalassoma duperrey*. Environ. Biol. Fishes 24, 117–136.

Nakamura, M., Mariko, T., Nagahama, Y., 1994. Ultrastructure and in vitro steroidogenesis of the gonads in the protandrous anemonefish *Amphiprion frenatus*. Jpn. J. Ichthyol. 41, 47–56.

Nakamura, M., Specker, J., Nagahama, Y., 1996. Innervation of steroid-producing cells in the ovary of tilapia *Oreochromis niloticus*. Zool. Sci. 13, 603–608.

Nakamura, M., Kobayashi, T., Chang, X.-T., Nagahama, Y., 1998. Gonadal sex differentiation in teleost fish. J. Exp. Biol. 281, 362–372.

Nakashima, Y., Kuwamura, T., Yogo, Y., 1995. Why be a both-ways sex changer? Ethology 101, 301–307.

Nakashima, Y., Kuwamura, T., Yogo, Y., 1996. Both-ways sex change in monogamous coral gobies, *Gobiodon* spp. Environ. Biol. Fishes 46, 281–288.

Nakayama, I., Foresti, F., Tewari, R., Schartl, M., Chourrout, D., 1994. Sex chromosome polymorphism and heterogametic males revealed by two cloned DNA probes in the ZW/ZZ fish *Leporinus elongatus*. Chromosoma 103, 31–39.

Nakayama, I., Biagi, C.A., Koide, N., Devlin, R.H., 1998. Identification of a sex-linked GH pseudogene in one of two species of Japanese salmon (*Oncorhynchus masou* and *O. rhodurus*). Aquaculture 173, 65–72.

Nakayama, C.M., Porto, J.I.R., Feldberg, E., 2000. Occurrence of two cytotypes in *Serrasalmus spilopleura* Kner, 1858 (Characiformes, Serrasalmidae) from the confluence region of the Negro and Solimoes rivers, Amazonas, Brazil. Acta Amazonica 30, 149–154.

Nakazono, A., Kusen, J.D., 1991. Protogynous hermaphroditism in the wrasse *Choerodon azurio*. Bull. Jpn. Soc. Sci. Fish. 57, 417–420.

Na-Nakorn, U., 1995. Comparison of cold and heat shocks to induce diploid gynogenesis in Thai walking catfish (*Clarias macrocephalus*) and performances of gynogens. Aquat. Living Resour. 8, 333–341.

Nanda, I., Feichtinger, W., Schmid, M., Schroeder, J.H., Zischler, H., Epplen, J.T., 1990. Simple repetitive

sequences are associated with differentiation of the sex chromosomes in the guppy fish. J. Mol. Evol. 30, 456–462.

Nanda, I., Schartl, M., Feichtinger, W., Epplen, J.T., Schmid, M., 1992. Early stages of sex chromosome differentiation in fish as analysed by simple repetitive DNA sequences. Chromosoma 101, 301–310.

Nanda, I., Schartl, M., Epplen, J.T., Feichtinger, W., Schmid, M., 1993. Primitive sex chromosomes in poeciliid fishes harbor simple repetitive DNA sequences. J. Exp. Zool. 265, 301–308.

Nanda, I., Volff, J.-N., Weis, S., Koerting, C., Froschauer, A., Schmid, M., Schartl, M., 2000. Amplification of a long terminal repeat-like element on the Y chromosome of the platyfish, *Xiphophorus maculatus*. Chromosoma 109, 173–180.

Nandeesha, M.C., Srikanth, G.K., Basavaraja, N., Varghese, T.J., Keshavanath, P., Shetty, H.P.C., Das, S.K., 1990. Effect of mibolerone on sex-reversal in *Oreochromis mossambicus*. Curr. Sci. 59, 748–750.

Naruse, K., Ijiri, K., Shima, A., Egami, N., 1985. The production of cloned fish in the medaka (*Oryzias latipes*). J. Exp. Zool. 236, 335–341.

Naruse, K., Fukamachi, S., Mitani, H., Kondo, M., Matsuoka, T., Kondo, S., Hanamura, N., Morita, Y., Hasegawa, K., Nishigaki, R., Shimada, A., Wada, H., Kusakabe, T., Suzuki, N., Kinoshita, M., Kanamori, A., Shima, A., 2000. A detailed linkage map of medaka, *Oryzias latipes*: comparative genomics and genome evolution. Genetics 154, 1773–1784.

Navas, J.M., Anglade, I., Bailhache, T., Pakdel, F., Breton, B., Jego, P., Kah, O., 1995. Do gonadotrophin-releasing hormone neurons express estrogen receptors in the rainbow trout? A double immunohistochemical study. J. Comp. Neurol. 363, 461–474.

Nayak, K., Khuda Bukhsh, A.R., 1988. Karyomorphology of a crocodile fish *Platycephalus tuberculatus* Platycephalidae Pisces. J. Inland Fish. Soc. India 20, 35–37.

Nayudu, P.L., 1979. Genetic studies of melanic color patterns and atypical sex determination in the guppy, *Poecilia reticulata*. Copeia 1979, 225–231.

Nelson, J.S., 1994. Fishes of the World. Wiley, New York, NY, 600 pp.

Nemtzov, S.C., 1985. Social control of sex change in the Red Sea razorfish *Xyrichtys pentadactylus* (Teleostei, Labridae). Environ. Biol. Fishes 14, 199–211.

Ngamvongchon, S., Kok, L.Y., Takashima, F., 1987. Changes in endocrine profiles and spermiation response in carp after LHRH analogue injection. Bull. Jpn. Soc. Sci. Fish. 53, 229–234.

Nilsson, E., Cloud, J., 1992. Rainbow trout chimeras produced by injection of blastomeres into recipient blastulae. Proc. Natl. Acad. Sci. U. S. A. 89, 9425–9428.

Nimrod, A.C., Benson, W.H., 1996. Environmental estrogenic effects of alkylphenol ethoxylates. Crit. Rev. Toxicol. 26, 335–364.

Nimrod, A.C., Benson, W.H., 1998. Reproduction and development of Japanese medaka following an early life stage exposure to xenoestrogens. Aquat. Toxicol. 44, 141–156.

Nishikawa, S., Sakamoto, K., 1978. Comparative studies on the chromosomes in Japanese fishes: 4. Somatic chromosomes of two lizardfishes. J. Shimonoseki Univ. Fish. 27, 113–117.

Nogusa, S., 1960. A comparative study of the chromosomes in fishes with particular consideration on taxonomy and evolution. Mem. Hyogo Univ. Agric. 3, 1–62.

Noichi, T., Kanbara, T., Subiyanto, T., Senta, T., 1991. Depth distribution of the percophid *Matsubaraea fusiforme* in Fukiagehama Beach Kyushu. Jpn. J. Ichthyol. 38, 245–248.

Nomura, T., Arai, K., Hayashi, T., Suzuki, R., 1998. Effect of temperature on sex ratios of normal and gynogenetic loach. Fish. Sci. 64, 753–758.

Norberg, B., Haux, C., 1988. An homologous radioimmunoassay for brown trout (*Salmo trutta*) vitellogenin. Fish Physiol. Biochem. 5, 59–68.

Nyman, L., Hammar, J., Gydemo, R., 1984. Lethal, sex-linked genes associated with homozygosity at the esterase-2 locus in Arctic char? Proceedings of the Third ISACF Workshop on Arctic Char, ISACF Inf. Ser., vol. 3, ISACF Inf. Ser., Tromsoe, Norway, pp. 125–130.

Oba, Y., Hirai, T., Yoshiura, Y., Yoshikuni, M., Kawauchi, H., Nagahama, Y., 1999. Cloning, functional characterization, and expression of a gonadotropin receptor cDNA in the ovary and testis of amagao salmon (*Oncorhynchus rhodurus*). Biochem. Biophys. Res. Commun. 263, 584–590.

Obi, A., 1988. Progeny sex ratios from intraspecific pair spawnings and random matings of *Oreochromis hornorum*. Niger. J. Appl. Fish Hydrobiol. 3, 35–37.

Obi, A., 1989. Progeny sex ratios from androgen-treated *Oreochromis hornorum*. J. Aquat. Sci. 4, 41–44.

Ochi, H., 1989. Mating behavior and sex change of the anemonefish *Amphiprion clarkii* in the temperate waters of Southern Japan. Environ. Biol. Fishes 26, 257–275.

O'Farrell, M.M., Pierce, R.E., 1989. The occurance of a gynandromorphic migratory trout, *Salmo trutta* L. J. Fish Biol. 34, 327.

Ohnishi, N., Yanagisawa, Y., Kohda, M., 1997. Sneaking by harem masters of the sandperch, *Parapercis snyderi*. Environ. Biol. Fishes 50, 217–223.

Ohno, S., 1970a. Evolution by Gene Duplication. Springer-Verlag, New York.

Ohno, S., 1970b. The enormous diversity in genome sizes of fish as a reflection nature's extensive experiments with gene duplication. Trans. Am. Fish. Soc. 99, 120–130.

Ojima, Y., 1985. Cited in Rishi, K.K. Current status of fish cytogenetics. In: Das and Jhingram (Eds.), Fish Genetics in India. Today and Tomorrow's Printers and Publishers, New Delhi, India. Chromosome Data Retrieval System.

Ojima, Y., Kikuno, T., 1986. A heteromorphic chromosome of *Beryx splendens*, Berycidae (Pisces). Proc. Jpn. Acad. 62, 317–320.

Ojima, Y., Ueda, H., Takai, A., 1984. Sex chromosome differentiation in *Parapercis sexfasciata* (Perciformes, Pisces). Proc. Jpn. Acad., Ser. B 60, 137–140.

Okada, H., 1973. Studies on sex differentiation of salmonidae: I. Effects of estrone on sex differentiation of the rainbow trout (*Salmo gairdneri irideus* Gibbons). Sci. Rep. Hokkaido Fish Hatchery 28, 11–21.

Okada, H., Matumoto, H., Yamazaki, F., 1979. Functional masculinization of genetic females in rainbow trout. Bull. Jpn. Soc. Sci. Fish. 45, 413–419.

Olin, T., Von Der Decken, A., 1987. Estrogen treatment and its implication on vitellogenin and myosin synthesis in salmon (*Salmo salar*). Physiol. Zool. 60, 346–351.

Olito, C., Brock, I., 1991. Sex reversal of rainbow trout creating an all-female population. Prog. Fish-Cult. 53, 41–44.

Oliveira, C., Foresti, F., Rigolino, M.G., Tabata, Y.A., 1995. Synaptonemal complex analysis in spermatocytes and oocytes of rainbow trout, *Oncorhynchus mykiss* (Pisces, Salmonidae): the process of autosome and sex chromosome synapsis. Chromosome Res. 3, 182–190.

Oliver, A.S., 1991. Hermaphrodite sex hormones: in vitro secretion of steroid hormones from the ovotestis of the teleost *Serranus subligarius*. Am. Zool. 31, 67A.

Oliver, A.S., 1997. Size and density dependent mating tactics in the simultaneously hermaphroditic seabass *Serranus subligarius* (Cope, 1870). Behaviour 134, 563–594.

Oliver, A.S., Thomas, P., Sullivan, C.V., 1995. Gametogenesis or behavior? The role of sex steroids in hermaphrodite reproduction. Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish. Fish Symposium 95, Austin, TX, USA, p. 275.

Olsen, L.C., Aasland, R., Fjose, A., 1997. A vasa-like gene in zebrafish identifies putative primordial germ cells. Mech. Dev., 66.

Omura, T., Morohashi, K., 1995. Gene regulation of steroidogenesis. J. Steroid Biochem. 53, 19–25.

Onozato, H., 1984. Diploidization of gynogenetically activated salmonid eggs using hydrostatic pressure. Aquaculture 43, 91–97.

Orzack, S., Sohn, J., Kallman, K., Levin, S., Johnston, R., 1980. Maintenance of the three sex chromosome polymorphism in the platyfish, *Xiphophorus maculatus*. Evolution 34, 663–672.

Oshiro, T., 1987. Sex ratios of diploid gynogenetic progeny derived from five different females of goldfish. Nissuishi/Bull. Jpn. Soc. Sci. Fish. 53, 1899.

Ota, K., Kobayashi, T., Ueno, K., Gojobori, T., 2000. Evolution of heteromorphic sex chromosomes in the order Aulopiformes. Gene 259, 25–30.

Owusu-Frimpong, M., Nijjhar, B., 1981. Induced sex reversal in *Tilapia nilotica* (Cichlidae) with methyl testosterone. Hydrobiologia 78, 157–160.

Ozouf Costaz, C., Teugels, G.G., Legendre, M., 1990. Karyological analysis of three strains of the african catfish *Clarias gariepinus* Clariidae used in aquaculture. Aquaculture 87, 271–278.

Pajuelo, J.G., Lorenzo, J.M., 1995. Biological parameters reflecting the current state of the exploited pink dentex *Dentex gibbosus* (Pisces: Sparidae) population off the Canary Islands. S. Afr. J. Mar. Sci. 16, 311–319.

Pakdel, F., Le Gac, F., Le Goff, P., Valotaire, Y., 1990. Full-length sequence and in vitro expression of rainbow trout estrogen receptor cDNA. Mol. Cell. Endocrinol. 71, 195–204.
Pakdel, F., Delaunay, F., Ducouret, B., Flouriot, G., Kern, L., Lazennec, G., Le Drean, Y., Petit, F., Salbert, G., Saligaut, D., Tujague, M., Valotaire, Y., 1997. Regulation of gene expression and biological activity of rainbow trout estrogen receptor. Fish Physiol. Biochem. 17, 1–6.
Pandey, N.L.W.S., 1997. Evidence of female heterogamety, B-chromosome and natural tetraploidy in the Asian catfish, *Clarias batrachus*, used in aquaculture. Aquaculture 149, 1–2.
Pandian, T.J., Sheela, S.G., 1995. Hormonal induction of sex reversal in fish. Aquaculture 138, 1–22.
Pandian, T.J., Varadaraj, K., 1990. Development of monosex female *Oreochromis mossambicus* broodstock by integrating gynogenetic technique with endocrine sex reversal. J. Exp. Zool. 255, 88–96.
Pannuti, A., Lucchesi, J.C., 2000. Recycling to remodel: evolution of dosage–compensation complexes. Curr. Opin. Genet. Dev. 10, 644–650.
Papoulias, D.M., Noltie, D.B., Tillitt, D.E., 2000. An in vivo model fish system to test chemical effects on sexual differentiation and development: exposure to ethinyl estradiol. Aquat. Toxicol. 48, 37–50.
Park, E.H., Kang, Y.S., 1979. Karyological confirmation of conspicuous ZW sex chromosomes in two species of *Pacific anguilloid* fishes (Anguilliformes: Teleostomi). Cytogenet. Cell Genet. 23, 33–38.
Park, I.S., Kim, H.B., Huh, H.T., Kim, S.C., 1993. Masculinization of masu salmon (*Oncorhynchus masou*) by treatments of 17-alpha-methyltestosterone. Ocean Res. 15, 29–36.
Park, I.S., Kim, H.S., Kim, E.S., Kim, J.H., Park, C.W., 1997. Cytogenetic analysis of river puffer, *Takifugu obscurus* (Teleostomi: Tetraodontiformes). Bull. Korean Fish. Soc. 30, 408–412.
Parmentier, H.K., Timmermans, L.P., 1985. The differentiation of germ cells and gonads during development of carp (*Cyprinus carpio* L.): a study with anticarpsperm monoclonal antibodies. J. Embryol. Exp. Morphol. 90, 13–32.
Parmentier, H.K., Timmermans, L.P., Egberts, E., 1984. Monoclonal antibodies against spermatozoa of the common carp (*Cyprinus carpio* L.): I. A study germ cell antigens in adult males and females. Cell Tissue Res. 236, 99–105.
Parsons, J.E., Thorgaard, G.H., 1985. Production of androgenetic diploid rainbow trout. J. Hered. 76, 177–181.
Pasmanik, M., Callard, G.V., 1988a. Changes in brain aromatase and 5 alpha-reductase activities correlate significantly with seasonal reproductive cycles in goldfish (*Carassius auratus*). Endocrinology 122, 1349–1356.
Pasmanik, M., Callard, G.V., 1988b. A high abundance androgen receptor in goldfish brain: characteristics and seasonal changes. Endocrinology 123, 1162–1171.
Pasmanik, M., Schlinger, B.A., Callard, G.V., 1988. In vivo steroid regulation of aromatase and 5 alpha-reductase in goldfish brain and pituitary. Gen. Comp. Endocrinol. 71, 175–182.
Passakas, T., 1981. Comparative studies on the chromosomes of the European eel (*Anguilla anguilla* L.) and the American eel (*Anguilla rostrata* Le Sueur). Folia Biol. 29, 41–58.
Patel, P.G., Angus, R.A., Bej, K., 1993. Simple repetitive DNA sequences associated with heterogametic sex in mosquitofish. J. Ala. Acad. Sci. 64, 93.
Patino, R., Thomas, P., 1990. Characterization of membrane receptor activity for 17 alpha, 20 beta, 21-trihydroxy-4-pregnen-3-one in ovaries of spotted seatrout (*Cynoscion nebulosus*). Gen. Comp. Endocrinol. 78, 204–217.
Patino, R., Davis, K.B., Schoore, J.E., Uguz, C., Struessmann, C.A., Parker, N.C., Simco, B.A., Goudie, C.A., 1996. Sex differentiation of channel catfish gonads: normal development and effects of temperature. J. Exp. Zool. 276, 209–218.
Patzner, R.A., Kaurin, G., 1997. Sexual differentiation in *Salaria* (equals *Blennius*) *pavo*. J. Fish Biol. 50, 887–894.
Pavlidis, M., Koumoundouros, G., Sterioti, A., Somarakis, S., Divanach, P., Kentouri, M., 2000. Evidence of temperature-dependent sex determination in the European sea bass (*Dicentrarchus labrax* L.). J. Exp. Zool. 287, 225–232.
Pechan, P., Wachtel, S.S., Reinboth, R., 1979. H–Y antigen in the teleost. Differentiation 14, 189–192.
Pechan, P., Shapiro, D.Y., Tracey, M., 1986. Increased H–Y antigen levels associated with behaviorally induced, female-to-male sex reversal in a coral-reef fish. Differentiation 31, 106–110.
Pendas, A.M., Moran, P., Freije, J.P., Garcia-Vazquez, E., 1994. Chromosomal mapping and nucleotide sequence of two tandem repeats of Atlantic salmon 5S rDNA. Cytogenet. Cell Genet. 67, 31–36.

Peng, C., Trudeau, V.L., Peter, R.E., 1993. Seasonal variation of neuropeptide Y actions on growth hormone and gonadotropin—II secretion in the goldfish effects of sex steroids. J. Neuroendocrinol. 5, 273–280.
Penman, D.J., Shah, M.S., Beardmore, J.A., Skibinski, D.O.F., 1987. Sex ratios of gynogenetic and triploid tilapia. Sel. Hybrid. Genet. Eng. Aquacult. 18, 18–19.
Pense, K., 1965. Cited in Rishi, K.K. Current status of fish cytogenetics. In: Das and Jhingram (Eds.), Fish genetics in India. Today and Tomorrow's Printers and Publishers, New Delhi, India. Rev. Suisse Zool. 49, 185.
Perez, L.E., Callard, I.P., 1993. Regulation of hepatic vitellogenin synthesis in the little skate *Raja erinacea* use of a homologous enzyme-linked immunosorbent assay. J. Exp. Zool. 266, 31–39.
Perez, F., Fuenzalida, A., Mendez, E., Cerisola, H., 1983. Myoid and Leydig-type interstitial cells are simultaneously innervated in the testes of the clingfish *Sicyases sanguineus* (Teleostei). Arch. Biol. Med. Exp. 16, 177.
Petersen, C.W., 1990. Variation in reproductive success and gonadal allocation in the simultaneous hermaphrodite *Serranus fasciatus*. Oecologia 83, 62–67.
Petersen, C.W., Fischer, E.A., 1996. Intraspecific variation in sex allocation in a simultaneous hermaphrodite: the effect of individual size. Evolution 50, 636–645.
Peterson, R.H., Benfey, T.J., McGeachy, S.A., Rommens, M., Richards, K., Harmon, P., 1996. Sex ratios of eels reared under two temperature regimes. Can. Tech. Rep. Fish. Aquat. Sci., 16 pp.
Petrino, T.R., Lin, Y.-W.P., Wallace, R.A., 1989. Steroidogenesis in *Fundulus heteroclitus*: I. Production of 17 alpha-hydroxy, 20 beta-dihydroprogesterone, testosterone, and 17 beta-estradiol by prematurational follicles in vitro. Gen. Comp. Endocrinol. 73, 147–156.
Peyon, P., Baloche, S., Burzawa-Gerard, E., 1997. Investigation into the possible role of androgens in the induction of hepatic vitellogenesis in the European eel: in vivo and in vitro studies. Fish Physiol. Biochem. 16, 107–118.
Pezold, F., 1984. Evidence for multiple sex chromosomes in the freshwater goby, *Gobionellus shufeldti* (Pisces: Gobiidae). Copeia 1984, 235–238.
Phelps, R.P., Cole, W., Katz, T., 1992. Effect of fluoxymesterone on sex ratio and growth of nile tilapia *Oreochromis niloticus* L. Aquacult. Fish. Manage. 23, 405–410.
Phillips, R.B., Ihssen, P.E., 1984. Chromosome banding difference between the X and Y chromosome of lake trout. Genetics 107, s82–s83.
Phillips, R.B., Ihssen, P.E., 1985. Identification of sex chromosomes in lake trout (*Salvelinus namaycush*). Cytogenet. Cell Genet. 39, 14–18.
Phillips, R.B., Zajicek, K.D., Utter, F.M., 1985. Q band chromosomal polymorphisms in chinook salmon (*Oncorhynchus tshawytscha*). Copeia 1985, 273–278.
Phillips, R.B. et al., 2001. Chromosome painting supports lack of homology among sex chromosomes in *Oncorhynchus*, *Salmo*, and *Salvelinus* (Salmonidae). Genetica 111, 119–123.
Piferrer, F., 2001. Endocrine sex control strategies for the feminization of teleost fish. Aquaculture 197, 229–281.
Piferrer, F., Donaldson, E.M., 1989. Gonadal differentiation in coho salmon, *Oncorhynchus kisutch*, after a single treatment with androgen or estrogen at different stages during ontogenesis. Aquaculture 77, 2–3.
Piferrer, F., Donaldson, E.M., 1991. Dosage-dependent differences in the effect of aromatizable and nonaromatizable androgens on the resulting phenotype of coho salmon (*Oncorhynchus kisutch*). Fish Physiol. Biochem. 9, 145–150.
Piferrer, F., Donaldson, E.M., 1992. The comparative effectiveness of the natural and a synthetic estrogen for the direct feminization of chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 106, 183–193.
Piferrer, F., Donaldson, E.M., 1994. Uptake and clearance of exogenous estradiol-17 beta and testosterone during the early development of coho salmon (*Oncorhynchus kisutch*), including eggs, alevins and fry. Fish Physiol. Biochem. 13, 219–232.
Piferrer, F., Baker, I.J., Donaldson, E.M., 1993. Effects of natural, synthetic, aromatizable, and nonaromatizable androgens in inducing male sex differentiation in genotypic female chinook salmon (*Oncorhynchus tshawytscha*). Gen. Comp. Endocrinol. 91, 59–65.
Piferrer, F., Benfey, T.J., Donaldson, E.M., 1994a. Gonadal morphology of normal and sex-reversed triploid and gynogenetic diploid coho salmon (*Oncorhynchus kisutch*). J. Fish Biol. 45, 541–553.
Piferrer, F., Carrillo, M., Zanuy, S., Solar, I.I., Donaldson, E.M., 1994b. Induction of sterility in coho salmon (*Oncorhynchus kisutch*) by androgen immersion before first feeding. Aquaculture 119, 409–423.

Piferrer, F., Zanuy, S., Carrillo, M., Solar, I.I., Devlin, R.H., Donaldson, E.M., 1994c. Brief treatment with an aromatase inhibitor during sex differentiation causes chromosomally female salmon to develop as normal, functional males. J. Exp. Zool. 270, 255–262.

Pinter, J., Thomas, P., 1995. Characterization of a progestogen receptor in the ovary of the spotted seatrout, *Cynoscion nebulosus*. Biol. Reprod. 52, 667–675.

Planas, J.V., Swanson, P., 1995. Maturation-associated changes in the response of the salmon testis to the steroidogenic actions of gonadotropins (GTH I and GTH II) in vitro. Biol. Reprod. 52, 697–704.

Pollock, B.R., 1985. The reproductive cycle of yellowfin bream, *Acanthopagrus australis* (Guenther), with particular reference to protandrous sex inversion. J. Fish Biol. 26, 301–311.

Polyakova, L.A., 1987. Induced gynogenesis in *Coregonus peled* Gm. (summary of development of the method). Sb. Nauchin. Tr. Gosniorkh. 261, 4–15.

Pongthana, N., Penman, D.J., Karnasuta, J., McAndrew, B.J., 1995. Induced gynogenesis in the silver barb (*Puntius gonionotus* Bleeker) and evidence for female homogamety. Aquaculture 135, 267–276.

Pongthana, N., Penman, D.J., Baoprasertkul, P., Hussain, M.G., Islam, M.S., Powell, S.F., McAndrew, B.J., 1999. Monosex female production in the silver barb (*Puntius gonionotus* Bleeker). Aquaculture 173, 247–256.

Porter, M.D., 1996. Effects of methyltestosterone on largemouth bass, *Micropterus salmoides*. J. Appl. Aquacult. 6, 39–46.

Porter, D.A., Fivizzani, A.J., 1983. Spontaneous occurrence of a synchronous hermaphrodite in the banded killifish, *Fundulus diaphanus* (Lesueur). J. Fish Biol. 22, 671–675.

Postlethwait, J.H., Johnson, S.L., Midson, C.N., Talbot, W.S., Gates, M., Ballinger, E.W., Africa, D., Andrews, R., Carl, T., Eisen, J.S., Horne, S., Kimmel, C.B., Hutchinson, M., Johnson, M., Rodriguez, A., 1994. A genetic linkage map for the zebrafish. Science 264, 699–703.

Potts, A.C., Phelps, R.P., 1995. Use of diethylstilbestrol and ethynylestradiol to feminize Nile tilapia *Oreochromis niloticus* (L.) in an outdoor environment. J. Appl. Ichthyol. 11, 111–117.

Pressley, P.H., 1981. Pair formation and joint territoriality in a simultaneous hermaphrodite: the coral reef fish *Serranus tigrinus*. Z. Tierpsychol. 56, 33–46.

Prodohl, P.A., Taggart, J.B., Ferguson, A., 1994. Single locus inheritance and joint segregation analysis of minisatellite (VNTR) DNA loci in brown trout (*Salmo trutta* L.). Heredity 73, 556–566.

Puckhaber, B., Horstgen-Schwak, G., 1996. Growth and gonadal development of triploid tilapia (*Oreochromis niloticus*). ICLARM Conference Proceedings, Makati City (Philippines). ICLARM, Makati: City, Philippines, pp. 377–382.

Pudney, J., Callard, G., 1984. Identification on Leydig-like cells in the testis of the dogfish *Squalus acanthias*. Anat. Rec. 209, 321–330.

Purdom, C.E., 1993. Genetics of fish breeding. Fish and Fisheries Series, vol. 8. Chapman & Hall, London.

Purvis, H., 1979. Variations in growth, age at transformation, and sex ratio of sea lampreys reestablished in chemically treated tributaries of the upper Great Lakes. Tech. Rep. GLFC 35, 1–36.

Quattro, J.M., Avise, J.C., Vrijenhoek, R.C., 1991. Molecular evidence for multiple origins of hybridogenetic fish clones (Poeciliidae: *Poeciliopsis*). Genetics 127, 391–398.

Quattro, J.M., Avise, J.C., Vrijenhoek, R.C., 1992a. Mode of origin and sources of genotypic diversity in triploid gynogenetic fish clones (*Poeciliopsis*: Poeciliidae). Genetics 130, 621–628.

Quattro, J.M., Avise, J.C., Vrijenhoek, R.C., 1992b. An ancient clonal lineage in the fish genus *Poeciliopsis* (Atheriniformes: Poeciliidae). Proc. Natl. Acad. Sci. U. S. A. 89, 348–352.

Quillet, E., Gaignon, J.L., 1990. Thermal induction of gynogenesis and triploidy in Atlantic salmon *Salmo salar* and their potential interest for aquaculture. Aquaculture 89, 351–364.

Quinitio, G.F., Caberoy, N.B., Reyes Jr., D.M., 1997. Induction of sex change in female *Epinephelus coioides* by social control. Isr. J. Aquacult. 49, 77–83.

Rab, P., 1984. Chromosome study of four poecilid fishes from Cuba. Folia Zool. 33, 229–234.

Rab, P., 1985. Karyotype of the Danube goby, *Proterorhinus marmoratus* (Pisces, Gobiidae). Folia Zool. (Brno) 34, 329–334.

Rab, P., Slechta, V., Flajshans, M., 1994. Cytogenetics, cytotaxonomy and Biochem. Genet. of Huchonine salmonids. Folia Zool. 43, 97–107.

Rao, H.N., Rao, G.P., 1983. Hormonal manipulation of sex in the common carp, *Cyprinus carpio* var. *communis* (Linnaeus). Aquaculture 35, 83–88.

Rasch, E., Monaco, P., Balsano, J., 1982. Cytophotometric and autoradiographic evidence for functional apomixis in a gynogenetic fish, *Poecilia formosa* and its related, triploid unisexuals. Histochemistry 73, 515–533.
Rasotto, M.B., 1992. Gonadal differentiation and the mode of sexuality in *Cobitis taenia* Teleostei Cobitidae. Copeia 1992, 223–228.
Ratanatham, S., Patinawin, S., 1979. Cytogenetic studies of Siamese fighting fish (*Betta splendens* Regan). J. Sci. Soc. Thailand 5, 17–26.
Ravaglia, M.A., Lo Nostro, F.L., Maggese, M.C., Guerrero, G.A., Somoza, G.M., 1997. Characterization of molecular variants of GnRH, induction of spermiation and sex reversal using salmon GnRH-A and domperidone in the protogynous diandric fish, *Synbranchus marmoratus* Bloch, (Teleostei, Synbranchidae). Fish Physiol. Biochem. 16, 425–436.
Raymond, C.S., Shamu, C.E., Shen, M.M., Seifert, K.J., Hirsch, B., Hodgkin, J., Zarkower, D., 1998. Evidence for evolutionary conservation of sex-determining genes. Nature 391, 691–695.
Reed, K.M., Phillips, R.B., 1997. Polymorphism of the nucleolus organizer region (NOR) on the putative sex chromosomes of Arctic char (*Salvelinus alpinus*) is not sex related. Chromosome Res. 5, 221–227.
Reed, K.M., Bohlander, S.K., Phillips, R.B., 1995. Microdissection of the Y chromosome and fluorescence in situ hybridization analysis of the sex chromosomes of lake trout, *Salvelinus namaycush*. Chromosome Res. 3, 221–226.
Refstie, T., Stoss, J., Donaldson, E.M., 1982. Production of all female coho salmon (*Oncorhynchus kisutch*) by diploid gynogenesis using irradiated sperm and cold shock. Aquaculture 29, 1–2.
Reinboth, R., 1975. Spontaneous and hormone-induced sex-inversion in wrasses (Labridae). Pubbl. Stn. Zool. Napoli 39, 550–573.
Reinboth, R., 1979. On steroidogenic pathways in ambisexual fishes. Proc. Indian Natl. Sci. Acad., Part B 45, 421–428.
Reinboth, R., Bruslé Sicard, S., 1997. Histological and ultrastructural studies on the effects of hCG on sex inversion in the protogynous teleost *Coris julis*. J. Fish Biol. 51, 738–749.
Reinboth, R., Becker, B., Latz, M., 1986. In vitro studies on steroid metabolism by gonadal tissues from ambisexual teleosts: II. Conversion of [^{14}C]androstenedione by the heterologous gonadal tissues of the protandric sea bream *Pagellus acarne* (Risso). Gen. Comp. Endocrinol. 62, 335–340.
Reinboth, R., Mayerova, A., Ebensperger, C., Wolf, U., 1987. The occurrence of serological H–Y antigen (Sxs antigen) in the diandric protogynous wrasse, *Coris julis* (L.) (Labridae, Teleostei). Differentiation 34, 13–17.
Reis-Henriques, M.A., Cruz, M.M., Pereira, J.O., 1997. The modulating effect of vitellogenin on the synthesis of 17 beta-estradiol by rainbow trout (*Oncorhynchus mykiss*) ovary. Fish Physiol. Biochem. 16, 181–186.
Ren, X., Yu, Q., Cui, J., Chang, Z., 1993. Fluorescence banding studies in fish chromosomes. Acta Genet. Sin. 20, 116–121.
Ridha, M.T., Lone, K.P., 1990. Effect of oral administration of different levels of 17-alpha methyltestosterone on the sex reversal growth and food conversion efficiency of the tilapia *Oreochromis spilurus* Guenther in brackish water. Aquacult. Fish. Manage. 21, 391–398.
Ridha, M.T., Lone, K.P., 1995. Preliminary studies on feminization and growth of *Oreochromis spilurus* (Guenther) by oral administration of 17 alpha-ethynyloestradiol in sea water. Aquacult. Res. 26, 479–482.
Riehl, R., 1991. Masculinization in a hermaphroditic female of the mosquitofish *Heterandria formosa*. Jpn. J. Ichthyol. 37, 374–380.
Rinchard, J., Kestemont, P., Heine, R., 1997. Comparative study of reproductive biology in single and multiple-spawner cyprinid fish: II. Sex steroid and plasma protein phosphorus concentrations. J. Fish Biol. 50, 169–180.
Rishi, K.K., 1973. Somatic karyotypes of three teleosts. Genen Phaenen 16, 101–107.
Rishi, K.K., 1975. Somatic and meiotic chromosomes of *Trichogaster fasciatus* (Bl. and Sch.) (Teleostei, Perciformes: Osphronemidae). Genen Phaenen 18, 49–53.
Rishi, K.K., 1976a. Karyotypic studies on four species of fish. Nucleus (Calcutta) 19, 95–98.
Rishi, K.K., 1976b. Mitotic and meiotic chromosomes of a teleost *Callichromis bimaculatus* (Bloch) with indications of male heterogamety. Cienc. Cult. 28, 1171–1173.
Rishi, K.K., 1981. Chromosomal studies on four cyprinid fishes. Int. J. Acad. Ichthyol. Modinagar 2, 1–4.
Rishi, K.K., Singh, J., 1983a. Karyological studies on two Indian estuarine catfishes, *Plotosus canius* Ham and *Pseudeutropius atherinoides* (Bloch). Caryologia 36, 139–144.

Rishi, K.K., Singh, J., 1983b. Chromosomal analysis of the Indian silurid, *Wallago attu* (Schneider) (Family: Siluridae). Chromosome Inf. Serv. 34, 10–11.
Rishi, K.K., Haobam, M.S., 1984. Karyotypes of three forms of fishes having high chromosome number. Int. J. Acad. Ichthyol. Modinagar 5, 1–2.
Rishi, K.K., Sharma, M.P., Mankotia, R., 1977. Somatic chromosomes of three Indian teleosts. Matsya 3, 6–9.
Roberts, D.E., Schlieder Jr., R.A., 1983. Induced sex inversion, maturation, spawning and embryogeny of the protogynous grouper, *Mycteroperca microlepis*. J. World Maric. Soc. 14, 639–649.
Robertson, D.R., 1972. Social control of sex reversal in a coral-reef fish. Science 177, 1007–1009.
Robertson, D.R., Justines, G., 1982. Protogynous hermaphroditism and gonochorism in four Caribbean reef gobies. Environ. Biol. Fishes 7, 137–142.
Robertson, D.R., Warner, R.R., 1978. Sexual patterns in the labroid fishes of the Western Caribbean: 2. The Parrotfishes (Scaridae). Smithson. Contrib. Zool. 255, 1–26.
Robertson, D.R., Reinboth, R., Bruce, R.W., 1982. Gonochorism, protogynous sex-change and spawning in three sparisonmatinine parrotfishes from the western Indian Ocean. Bull. Mar. Sci. 32, 868–879.
Roblin, C., Bruslé, J., 1983. Gonadal ontogenesis and sex differentiation in the sea bass, *Dicentrarchus labrax*, under fish-farming conditions. Reprod., Nutr., Dev. 23, 115–127.
Rodgers-Gray, T.P., Jobling, S., Kelly, C., Morris, S., Brighty, G., Waldock, M.J., Sumpter, J.P., Tyler, C.R., 2001. Exposure of juvenile roach (*Rutilus rutilus*) to treated sewage effluent induces dose-dependent and persistent disruption in gonadal duct development. Environ. Sci. Technol. 35, 462–470.
Rodriguez, L., Carrillo, M., Sorbera, L.A., Soubrier, M.A., Mananos, E., Holland, M.C.H., Zohar, Y., Zanuy, S., 2000. Pituitary levels of three forms of GnRH in the male European sea bass (*Dicentrarchus labrax*, L.) during sex differentiation and first spawning season. Gen. Comp. Endocrinol. 120, 67–74.
Roemer, U., Beisenherz, W., 1996. Environmental determination of sex in Apistogramma (Cichlidae) and two other freshwater fishes (Teleostei). J. Fish Biol. 48, 714–725.
Rokicki, J., Kulikowski, M., 1994. Occurrence of male *Carassius auratus* gibelio (Bloch, 1783) in Poland. Przegl. Zool. 38, 89–92.
Romanov, A.A., Altuf'ev, Y., 1992. Extraregional histogenesis of sexual cells in the Caspian Sea sturgeons. J. Ichthyol. 32, 145–154.
Romanov, A.A., Altuf'yev, Y., 1993. Ectopic histogenesis of sexual cells of Caspian Sea sturgeons. J. Ichthyol. 33, 140–150.
Roncarati, A., Melotti, P., Mordenti, O., Gennari, L., 1997. Influence of stocking density of European eel (*Anguilla anguilla*, L.) elvers on sex differentiation and zootechnical performances. J. Appl. Ichthyol. 13, 131–136.
Rosa Molinar, E., Fritzsch, B., Hendricks, S.E., 1996. Organizational-activational concept revisited: sexual differentiation in an atherinomorph teleost. Horm. Behav. 30, 563–575.
Rosenstein, S., Hulata, G., 1992. Sex reversal in the genus *Oreochromis*: 1. Immersion of eggs and embryos in oestrogen solutions is ineffective. Aquacult. Fish. Manage. 23, 669–678.
Rosenstein, S., Hulata, G., 1994. Sex reversal in the genus *Oreochromis*: optimization of feminization protocol. Aquacult. Fish. Manage. 25, 329–339.
Ross, R., 1978. Reproductive behavior of the anemonefish *Amphiprion melanopus* on Guam. Copeia 1978, 103–107.
Ross, R.M., 1981. Experimental evidence for stimulation and inhibition of sex change in the saddleback wrasse *Thalassoma duperrey.* Pac. Sci. 35, 275.
Ross, R.M., 1987. Sex-change linked growth acceleration in a coral-reef fish, *Thalassoma duperrey.* J. Exp. Zool. 244, 455–461.
Ross, R.M., 1990. The evolution of sex-change mechanisms in fishes. Environ. Biol. Fishes 29, 81–93.
Ross, R.M., Losey, G.S., Diamond, M., 1983. Sex change in a coral-reef fish: dependence of stimulation and inhibition on relative size. Science 217, 574–576.
Ross, R.M., Hourigan, T.F., Lutnesky, M.M.F., Singh, I., 1990. Multiple simultaneous sex changes in social groups of a coral-reef fish. Copeia 1990, 427–433.
Rossi, A.R., Crosetti, D., Gornung, E., Sola, L., 1996. Cytogenetic analysis of global populations of *Mugil cephalus* (striped mullet) by different staining techniques and fluorescent in situ hybridization. Heredity 76, 77–82.

Rothbard, S., Moav, B., Yaron, Z., 1987. Changes in steroid concentrations during sexual ontogenesis in tilapia. Aquaculture 61, 59–74.
Rothbard, S., Rubinshtein, I., David, L., Shelton, W.L., 1999. Ploidy manipulations aimed to produce androgenetic Japanese ornamental (koi) carp, *Cyprinus carpio* L. Isr. J. Aquacult.-Bamidgeh. 51, 26–39.
Rowland, S.J., Snape, R., 1994. Labile protogynous hermaphroditism in the black bream, *Acanthopagrus butcheri* (Munro) (Sparidae). Proc. Linn. Soc. N. S. W. 114, 225–232.
Ruan, H., Wu, G., Huang, R., 1996. Induced sex reversal of black sea bream, (*Sparus macrocephalus*). Stud. Mar. Sin., 151–161.
Rubin, D.A., 1985. Effect of pH on sex ratio in cichlids and a poeciliid (Teleostei). Copeia 1985, 233–235.
Rudek, Z.Z., 1974. Cited in Rishi, K.K. Current status of fish cytogenetics. In: Das and Jhingram (Eds.), Fish Genetics in India. Today and Tomorrow's Printers and Publishers, New Delhi, India. Folia Biol. 22, 211.
Ruiz Carus, R., 1983. A karyotypic study of *Epinephelus guttatus* (Linnaeus) and *Thalassoma bifasciatum* (Bloch) (Serranidae and Labridae–Pisces). (abstract of MS thesis). Contribuciones. Department of Marine Sciences, University of Puerto Rico, Mayagüez, pp. 254–255.
Ryan, M.J., Pease, C.M., Morris, M.R., 1992. A genetic polymorphism in the swordtail *Xiphophorus nigrensis*: testing the prediction of equal fitnesses. Am. Nat. 139, 21–31.
Ryazantseva, M.V., Sakun, O.F., 1980. The sex cells and development of the early ontogeny of the carp, *Cyprinus carpio*. J. Ichthyol. 20, 114–122.
Sadovy, Y., Colin, P.L., 1995. Sexual development and sexuality in the Nassau grouper. J. Fish Biol. 46, 961–976.
Sadovy, Y., Donaldson, T.J., 1995. Sexual pattern of *Neocirrhites armatus* (Cirrhitidae) with notes on other hawkfish species. Environ. Biol. Fishes 42, 143–150.
Sadovy, Y., Shapiro, D.Y., 1987. Criteria for the diagnosis of hermaphroditism in fishes. Copeia 1987, 136–156.
Sadovy, Y., Rosario, A., Romain, A., 1994. Reproduction in an aggregating grouper, the red hind, *Epinephelus guttatus*. Environ. Biol. Fishes 41, 269–286.
Saga, T., Oota, Y., Nozaki, M., Swanson, P., 1993. Salmonid pituitary gonadotrophs: III. Chronological appearance of GTH I and other adenohypophysial hormones in the pituitary of the developing rainbow trout (*Oncorhynchus mykiss irideus*). Gen. Comp. Endocrinol. 92, 233–241.
Saitoh, K., 1989. Multiple sex-chromosome system in a loach fish. Cytogenet. Cell Genet. 52, 62–64.
Sakai, Y., 1997. Alternative spawning tactics of female angelfish according to two different contexts of sex change. Behav. Ecol. 8, 372–377.
Sakai, Y., Kohda, M., 1997. Harem structure of the protogynous angelfish, *Centropyge ferrugatus* (Pomacanthidae). Environ. Biol. Fishes 49, 333–339.
Sakai, N., Iwamatsu, T., Yamauchi, K., Nagahama, Y., 1987. Development on the steroidogenic capacity of medaka (*Oryzias latipes*) ovarian follicles during vitellogenesis and oocyte maturation. Gen. Comp. Endocrinol. 66, 333–342.
Sakai, N., Iwamatsu, T., Yamauchi, K., Suzuki, N., Nagahama, Y., 1988. Influence of follicular development on steroid production in the medaka (*Oryzias latipes*) ovarian follicle in response to exogenous substrates. Gen. Comp. Endocrinol. 71, 516–523.
Sakai, N., Tanaka, M., Takahashi, M., Fukada, S., Mason, J., Nagahama, Y., 1994. Ovarian 3 beta-hydroxysteroid dehydrogenase/Delta super(5–4)-isomerase of rainbow trout: its cDNA cloning and properties of the enzyme expressed in mammalian cells. FEBS Lett. 350, 309–313.
Sakaizumi, M., Shimizu, Y., Matsuzaki, T., Kurita, J., Oshiro, T., Hamaguchi, S., 1991. Non-reductional diploid eggs produced by females of interspecific hybrids between *Oryzias latipes* and *O. curvinotus*. Zool. Sci. 8, 1126.
Salvadori, S., Cau, A., Deiana, A.M., Paci, S., Mezzanotte, R., 1989. Preliminary data on the use of restriction enzymes to study *Muraena helena* chromosomes. Oebalia 15, 769–777.
Sanchez, S.F.A.S., 1996. Karyotypic studies and cytotaxonomic considerations on *Callichthys callichthys* (Pisces, Siluroidei) from Argentina. Cytologia 61, 247–252.
Sanchez, S., Jorge, L.C., 1999. A new report of the ZZ/ZW sex chromosome system in the genus *Triportheus* (Pisces, Triporthinae). Cytologia 64, 395–400.
Sangalang, G.B., Freeman, H.C., 1988. In vitro biosynthesis of 17 alpha,20 alpha,20 beta-dihydroxy-4-pegnen-3-

one by the ovaries, testes, and head kidneys of the Atlantic salmon *Salmo salar*. Gen. Comp. Endocrinol. 69, 406–415.

Santacruz, H., Vriz, S., 1996. Regional distribution of maternal mRNA Sox-19 in the zebrafish early embryo. Biol. Cell 88, 153–155.

Sasaki, T., Sakamoto, K., 1977. Karyotype of the rockfish, *Sebastes taczanowskii* Steindachner. Chromosome Inf. Serv. 22, 7–8.

Sato, T., et al., 2001. Gene-centromere mapping of medaka sex chromosomes using triploid hybrids between *Oryzias latipes* and *O. luzonensis*. Genetica 111, 71–75.

Satoh, N., 1974. An ultrastructural study of sex differentiation in the teleost *Oryzias latipes*. J. Embryol. Exp. Morphol. 32, 192–215.

Satoh, N., Egami, N., 1972. Sex differentiation of germ cells in the teleost, *Oryzias latipes*, during normal embryonic development. J. Embryol. Exp. Morphol. 28, 385–395.

Saxena, O.P., Bhatia, M.K., 1977. Intersexuality in the fresh water teleost, *Heteropneustes fossilis*. Curr. Sci. 46, 307.

Scavone, M.D.P., Julio Jr., H.F., 1995. Cytogenetics analysis and heterochromatin distribution in ZZ/ZW sex chromosomes of the mailed catfish *Loricariichthys platymetopon* (Loricariidae: Siluriformes). Rev. Bras. Genet. 18, 31–35.

Schartl, M., 1988. A sex chromosomal restriction-fragment-length marker linked to melanoma-determining Tu loci in *Xiphophorus*. Genetics 119, 679–685.

Schartl, M., 1990. Homology of melanoma-inducing loci in the genus *Xiphophorus*. Genetics 126, 1083–1091.

Schartl, M., Adam, D., 1992. Molecular cloning, structural characterization, and analysis of transcription of the melanoma oncogene of *Xiphophorus*. Pigm. Cell Res., 173–180.

Schartl, M., Nanda, I., Schlupp, I., Wilde, B., Epplen, J.T., Schmid, M., Parzefall, J., 1995. Incorporation of subgenomic amounts of DNA as compensation for mutational load in a gynogenetic fish. Nature 373, 68–71.

Scheerer, P., Thorgaard, G., Allendorf, F., Knudsen, K., 1986. Androgenetic rainbow trout produced from inbred and outbred sperm sources show similar survival. Aquaculture 57, 289–298.

Scheerer, P.D., Thorgaard, G.H., Allendorf, F.W., 1991. Genetic analysis of androgenetic rainbow trout. J. Exp. Zool. 260, 382–390.

Schlenk, D., Ronis, M.J.J., Miranda, C.L., Buhler, D.R., 1993. Channel catfish liver monooxygenases: immunological characterization of constitutive cytochromes *P*450 and the absence of active flavin-containing monooxygenases. Biochem. Pharmacol. 45, 217–221.

Schlupp, I., Nanda, I., Dobler, M., Lamatsch, D.K., Epplen, J.T., Parzefall, J., Schmid, M., Schartl, M., 1998. Dispensable and indispensable genes in an ameiotic fish, the Amazon molly *Poecilia formosa*. Cytogenet. Cell Genet. 80, 193–198.

Schmelzing, T.O., Gall, G.A.E., 1991. Use of 17-alpha methyltestosterone to sex inverse gynogenic female rainbow trout. J. Appl. Ichthyol. 7, 120–128.

Scholz, S., Gutzeit, H.O., 2000. 17-alpha-Ethinylestradiol affects reproduction, sexual differentiation and aromatase gene expression of the medaka (*Oryzias latipes*). Aquat. Toxicol. 50, 363–373.

Schreibman, M.P., Kallman, K.D., 1978. The genetic control of sexual maturation in the teleost, *Xiphophorus maculatus* (Poeciliidae); a review. Ann. Biol. Anim., Biochim., Biophys. 18, 957–962.

Schreibman, M.P., Berkowitz, E.J., van den Hurk, R., 1982. Histology and histochemistry of the testis and ovary of the platyfish, *Xiphophorus maculatus*, from birth to sexual maturity. Cell Tissue Res. 224, 781–787.

Schultz, R.J., 1967. Gynogenesis and triploidy in the viviparous fish *Poeciliopsis*. Science 157, 1564–1567.

Schultz, R.J., 1969. Hybridization, unisexuality, and polyploidy in the teleost *Poeciliopsis* (Poeciliidae) and other vertebrates. Am. Nat. 103, 605–619.

Schultz, R.J., 1971. Special adaptive problems associated with unisexual fish. Am. Zool. 11, 351–360.

Schultz, R.J., 1973. Unisexual fish: laboratory synthesis of a "species". Science 179, 180–181.

Schulz, R., 1986. in vitro metabolism of steroid hormones in the liver and in blood cells of male rainbow trout (*Salmo gairdneri* Richardson). Gen. Comp. Endocrinol. 64, 312–319.

Schultz, R.J., 1993. Genetic regulation of temperature-mediated sex ratios in the livebearing fish *Poeciliopsis lucida*. Copeia 1993, 1148–1151.

Schultz, H., 1996. Drastic decline of the proportion of males in the roach (*Rutilus rutilus* L.) population of

Bautzen Reservoir (Saxony, Germany): results of direct and indirect effects of biomanipulation. Limnologica 26, 153–164.

Schulz, R., Bluem, V., 1990. Steroid secretion of rainbow trout testis in vitro: variation during the reproductive cycle. Gen. Comp. Endocrinol. 80, 189–198.

Schulz, R., Bluem, V., 1991. Extragonadal 17 beta-hydroxysteroid dehydrogenase activity in rainbow trout. Gen. Comp. Endocrinol. 82, 197–205.

Schulz, R.W., Andriske, M., Lembke, P.J., Blum, V., 1992. Effect of salmon gonadotropic hormone on sex steroids in male rainbow trout: plasma levels and testicular secretion in vitro. J. Comp. Physiol., B 162, 224–230.

Schulz, R.W., Van Der Corput, L., Janssen-Dommerholt, J., Goos, H.J.T., 1994. Sexual steroids during puberty in male African catfish (*Clarias gariepinus*): serum levels and gonadotropin-stimulated testicular secretion in vitro. J. Comp. Physiol., B 164, 195–205.

Schwarz, A.L., Smith, C.L., 1990. Sex change in the damselfish *Dascyllus reticulatus* (Richardson) (Perciformes: Pomacentridae). Bull. Mar. Sci. 46, 790–798.

Scott, A.P., MacKenzie, D.S., Stacey, N.E., 1984. Endocrine changes during natural spawning in the white sucker, *Catostomus commersoni*: II. Steroid hormones. Gen. Comp. Endocrinol. 56, 349–359.

Scott, A.G., Penman, D.J., Beardmore, J.A., Skibinski, D.O.F., 1989. The "YY" supermale in *Oreochromis niloticus* (L.) and its potential in aquaculture. Aquaculture 78, 3–4.

Seeb, J.E., Thorgaard, G.H., Utter, F.M., 1988. Survival and allozyme expression in diploid and triploid hybrids between chum, chinook, and coho salmon. Aquaculture 72, 31–48.

Sehgal, G.K., Saxena, P.K., 1995a. Effect of 17-alpha-methyltestosterone on sex composition, growth and flesh composition in common carp, *Cyprinus carpio* (L.) communis. Indian J. Exp. Biol. 33, 169–172.

Sehgal, G.K., Saxena, P.K., 1995b. Effect of 5-alpha-dihydrotestosterone on sex composition, growth and flesh composition in common carp, *Cyprinus carpio* (Linn.). Proc. Indian Natl. Sci. Acad., Part B 61, 431–436.

Sehgal, G.K., Saxena, P.K., 1997a. Effect of estradiol-17 beta on sex composition, growth and flesh composition in common crap, *Cyprinus carpio* communis (Linn.). Indian J. Exp. Biol. 35, 498–501.

Sehgal, G.K., Saxena, P.K., 1997b. Effect of estrone on sex composition, growth and flesh composition in common carp, *Cyprinus carpio* communis (Linn.). J. Aquacult. Trop. 12, 289–295.

Severin, S.O., 1979. The karyotype of the European grayling, *Thymallus thymallus*. J. Ichthyol. 19, 44–49.

Shah, M.S., 1988. Female homogamety in tilapia (*Oreochromis niloticus*) revealed by gynogenesis. Asian Fish. Sci. 1, 215–219.

Shapiro, D.Y., 1979. On the causes of sex reversal in coral reef fish. Proc. Assoc. Isl. Mar. Lab. Caribb. 14, 22.

Shapiro, D.Y., 1980. Serial female sex changes after simultaneous removal of males from social groups of a coral reef fish. Science 209, 1136–1137.

Shapiro, D., 1981a. Size, maturation and the social control of sex reversal in the coral reef fish *Anthias squamipinnis*. J. Zool. 193, 105–128.

Shapiro, D.Y., 1981b. Size, maturation and the social control of sex reversal in the coral reef fish *Anthias squamipinnis*. J. Zool. 193, 105–128.

Shapiro, D.Y., 1981c. Sequence of coloration changes during sex reversal in the tropical marine fish *Anthias squamipinnis* (Peters). Bull. Mar. Sci. 31, 383–398.

Shapiro, D.Y., 1986. Intra-group home ranges in a female-biased group of sex-changing fish. Anim. Behav. 34, 865–870.

Shapiro, D.Y., 1988. Variation of group composition and spatial structure with group size in a sex-changing fish. Anim. Behav. 36, 140–149.

Shapiro, D.Y., 1990. Sex-changing fish as a manipulable system for the study of the determination, differentiation, and stability of sex in vertebrates. J. Exp. Zool., Suppl. 4, 132–136.

Shapiro, D.Y., 1992. Plasticity of gonadal development and protandry in fishes. J. Exp. Zool. 261, 194–203.

Shapiro, D.Y., Rasotto, M.B., 1993. Sex differentiation and gonadal development in the diandric, protogynous wrasse, *Thalassoma bifasciatum* (Pisces, Labridae). J. Zool. 230, 231–245.

Shapiro, D.Y., Sadovy, Y., McGehee, M.A., 1993a. Periodicity of sex change and reproduction in the red hind, *Epinephelus guttatus*, a protogynous grouper. Bull. Mar. Sci. 53, 1151–1162.

Shapiro, D.Y., Sadovy, Y., McGehee, M.A., 1993b. Size composition and spatial structure of the annual spawning aggregation of the red hind *Epinephelus guttatus* Pisces Serranidae. Copeia 1993, 399–406.

Shapiro, D.Y., Garcia Moliner, G., Sadovy, Y., 1994. Social system of an inshore stock of the red hind grouper, *Epinephelus guttatus* (Pisces: Serranidae). Environ. Biol. Fishes 41, 415–422.
Sharma, O.P., Tripathi, N.K., 1982. Somatic and germ cell chromosomes of *Gambusia affinis* holbrooki. Chromosome Inf. Serv. 32, 24–25.
Sharma, O.P., Tripathi, N.K., 1984. Studies on the chromosomes of *Nandus nandus* and *Badis badis* from the J and K state, India. Cytologia 49, 73–79.
Sharma, O.P., Tripathi, N.K., 1988. Female heterogamety in two Teleostean fishes. Cytologia 53, 81–86.
Sharma, O.P., Tripathi, N.K., Agrawal, A., Tripathi, S., 1990. Karyotypic diversity in genus *Puntius* Cyprinidae Pisces. Nucleus 33, 81–83.
Sharma, K.K., Sharma, O.P., Tripathi, N.K., 1998. Female heterogamety in *Danio rerio* (Cypriniformes: Cyprinidae). Proc. Natl. Acad. Sci., India, Sect. B 68, 123–126.
Sheaves, M., 1995. Large lutjanid and serranid fishes in tropical estuaries: are they adults or juveniles? Mar. Ecol.: Prog. Ser. (Oldendorf) 129, 1–3.
Shelenkova, N., 1987. A study of karyotypes of two Pacific salmon species, *Oncorhynchus nerka* Walbaum and *O. kisutch* W., by the C-banding method. Tsitologiya 29, 95–100.
Shelton, W.L., 1986. Broodstock development for monosex production of grass carp. Aquaculture 57, 1–4.
Shelton, W.L., Rodriguez-Guerrero, D., Lopez-Macias, J., 1981. Factors affecting androgen sex reversal of *Tilapia aurea*. Aquaculture 25, 59–65.
Shelton, W.L., Wanniasingham, V., Hiott, A.E., 1995. Ovarian differentiation in common carp (*Cyprinus carpio*) in relation to growth rate. Aquaculture 137, 1–4.
Shen, S.C., Liu, C.H., 1976. Ecological and morphological study of the fish-fauna from the waters around Taiwan and its adjacent islands: 17. A study of sex reversal in a pomacanthid fish, *Genicanthus semifasciatus* (Kamohara). Acta Oceanogr. Taiwan. 6, 140–150.
Shen, J., Fan, Z., Wang, G., 1983. Karyotype studies of male triploid crucian carp (*Fangzheng crucian* carp) in Heilongjiang. Acta Genet. Sin. 10, 133–136.
Shepherd, G.R., Idoine, J.S., 1993. Length-based analyses of yield and spawning biomass per recruit for Black Sea bass *Centropristis striata* a protogynous hermaphrodite. U.S. Natl. Mar. Fish. Serv. Fish. Bull.-NOAA 91, 328–337.
Shibata, N., Hamaguchi, S., 1988. Evidence for the sexual bipotentiality of spermatogonia in fish, *Oryzias latipes*. J. Exp. Zool. 245, 71–77.
Shibuno, T., Gushima, K., Kakuda, S., 1993a. Female spawning migrations of the protogynous wrasse *Halichoeres marginatus*. Jpn. J. Ichthyol. 39, 357–362.
Shibuno, T., Chiba, I., Gushima, K., Kakuda, S., Hashimoto, H., 1993b. Reproductive behavior of the wrasse, *Halichoeres marginatus*, at Kuchierabu-jima. Jpn. J. Ichthyol. 40, 351–359.
Shibuno, T., Chiba, I., Hashimoto, H., Gushima, K., 1994. Reproductive behavior of the wrasse, *Thalassoma lutescens*, at Kuchierabu-jima. J. Fac. Appl. Biol. Sci., Hiroshima Univ. 33, 43–50.
Shibuno, T., Chiba, I., Hashimoto, H., Gushima, K., 1995. Acquisition of mating territories in the wrasse, *Thalassoma lutescens*. J. Fac. Appl. Biol. Sci., Hiroshima Univ. 34, 179–183.
Siau, Y., 1994. Population structure, reproduction and sex-change in a tropical East Atlantic grouper. J. Fish Biol. 44, 205–211.
Siau, Y., Bouain, A., 1994a. Variations in spawning of two species of coastal hermaphrodite fishes, genus *Serranus*, related to their bathymetric distribution. Oebalia 20, 1–20.
Siau, Y., Boauain, A., 1994b. Preliminary indications on growth and reproduction in the protogynous grouper *Mycteroperca rubra* (Pisces, Serranidae). J. Afr. Zool. 108, 353–359.
Simpson, E., Scott, D., Chandler, P., 1997. The male-specific histocompatibility antigen, H–Y: a history of transplantation, immune response genes, sex determination and expression cloning. Annu. Rev. Immunol. 15, 39–61.
Singh, H., 1993. Effects of malathion on steroidogenesis and sex reversal in *Monopterus albus*. Respir. Mar. Org. Pollut. 35, 1–2.
Sishi, K.K., Singh, J., 1983. Chromosomes of *Notopterus notopterus* (Pallas) (Notopteridae: Clupeiformes). Chromosome Inf. Serv. 34, 9–10.
Smith, C.L., 1975. The evolution of hermaphroditism in fish. In: Reinboth, R. (Ed.), Intersexuality in the Animal Kingdom. Springer, Berlin, pp. 295–310.

Smith, J.S., Thomas, P., 1990. Binding characteristics of the hepatic estrogen receptor of the spotted seatrout *Cynoscion nebulosus*. Gen. Comp. Endocrinol. 77, 29–42.

Snelson Jr., F., Wetherington, J., 1980. Sex ratio in the sailfin molly, *Poecilia latipinna*. Evolution 34, 308–319.

Snowberger, E.A., Stegeman, J.J., 1987. Patterns and regulation of estradiol metabolism by hepatic microsomes from two species of marine teleosts. Gen. Comp. Endocrinol. 66, 256–265.

Sobhnana, K.S., Nandeesha, M.C., 1994. Standardization of mibolerone dosage for production of female-free common carp (*Cyprinus carpio* var. communis L.) and the impact of the hormone of growth and flesh composition. J. Aquacult. Trop. 9, 133–139.

Sofradzija, A., Vukovic, T., 1979. Chromosome complement of *Nemachilus barbatulus* (Linnaeus, 1758) Cobitidae, Pisces. Acta Biol. Iugosl. 11, 43–49.

Sohoni, P., Sumpter, J.P., 1998. Several environmental oestrogens are also anti-androgens. J. Endocrinol. 158, 327–339.

Sola, L., Monaco, P.J., Rasch, E.M., 1990. Cytogenetics of bisexual/unisexual species of *Poecilia*: I. C-bands, Ag-NOR polymorphisms, and sex chromosomes in three populations of *Poecilia latipinna*. Cytogenet. Cell Genet. 53, 148–154.

Sola, L., Rossi, A.R., Iaselli, V., Rasch, E.M., Monaco, P.J., 1992a. Cytogenetics of bisexual/unisexual species of *Poecilia*: II. Analysis of heterochromatin and nucleolar organizer regions in *Poecilia mexicana* mexicana by C-banding and DAPI, quinacrine, chromomycin A3, and silver staining. Cytogenet. Cell Genet. 60, 229–235.

Sola, L., Iaselli, V., Rossi, A.R., Rasch, E.M., Monaco, P.J., 1992b. Cytogenetics of bisexual/unisexual species of *Poecilia*: III. The karyotype of *Poecilia formosa*, a gynogenetic species of hybrid origin. Cytogenet. Cell Genet. 60, 3–4.

Solar, I., Donaldson, E.M., 1985. Studies on genetic and hormonal sex control in a domesticated rainbow trout: 2. Use of methyltestosterone for masculinization and sterilization in cultured rainbow trout (*Salmo gairdneri* Richardson). Can. Tech. Rep. Fish. Aquat. Sci. 1380, 1–13.

Solar, I.I., Donaldson, E.M., Douville, D., 1991. A bibliography of gynogenesis and androgenesis in fish (1913–1989). Can. Tech. Rep. Fish. Aquat. Sci. 1788, 1–45.

Solar, I.I., Donaldson, E.M., Charles, J., 1994. The effect of three estrogens on the direct feminization of chinook salmon (*Oncorhynchus tshawytscha*). Can. Tech. Rep. Fish. Aquat. Sci 1955, 1–11.

Somoza, G.M., Peter, R.E., 1991. Effects of serotonin on gonadotropin and growth hormone release from in vitro perifused goldfish pituitary fragments. Gen. Comp. Endocrinol. 82, 103–110.

Son, J.K., 1991. Histological studies of sex reversal. Korean J. Anim. Sci. 33, 339–341.

Sorensen, P.W., Goetz, F.W., 1993. Pheromonal and reproductive function of F prostaglandins and their metabolites in teleost fish. J. Lipid Mediators 6, 385–393.

Soto, C.G., Noakes, D.L.G., 1994. Coloration and gender in the hermaphroditic fish *Rivulus marmoratus* Poey (Teleostei: Rivulidae). Ichthyol. Explor. Freshwaters (Munchen) 5, 79–90.

Soto, C.G., Leatherland, J.F., Noakes, D.L.G., 1992. Gonadal histology in the self-fertilizing hermaphroditic fish *Rivulus marmoratus* Pisces Cyprinodontidae. Can. J. Zool. 70, 2338–2347.

Souza, I.L., Moreira Filho, O., Antonio Carlos Bertollo, L., 1995. Karyotypic characterization of *Aphyocharax difficilis* (Pisces, characidae): C-banding, Ag–NORs and occurrence of diplochromosomes. Cytobios 83, 33–39.

Sower, S., Dickhoff, W., Flagg, T., Mighell, J., Mahnken, C., 1984. Effects of estradiol and diethylstilbesterol on sex reversal and mortality in Atlantic salmon (*Salmo salar*). Aquaculture 43, 75–81.

Spink, D.C., Eugster, H.-P., Licoln, D.W., Scheutz, J.D., Scheutz, E.G., Johnson, J.A., Kaminsky, L.S., Gierthy, J.F., 1992. 17beta-estradiol hydroxylation catalyzed by human cytochrome *P*4501A1: a comparison of the activities induced by 2,3,7,8-tetrachlorodibenzeno-*p*-dioxin in MCF-7 cells with those from the heterologous expression of the cDNA. Arch. Biochem. Biophys. 293, 342–348.

Springer, V., Lavett Smith, C., Fraser, T., 1978. *Anisochromis straussi*, new species of protogynous hermaphroditic fish, and synonymy of Anisochromidae, Pseudoplesiopidae, and Pseudochromidae. Smithson. Contrib. Zool. 252, 1–15.

Srivastava, S.J., Singh, R., 1989. Testis–ova in the murrel *Channa punctatus* Bloch. Naturalia 14, 63–66.

St. Mary, C.M., 1993. Novel sexual patterns in two simultaneously hermaphroditic gobies *Lythrypnus dalli* and *Lythrypnus zebra*. Copeia 1993, 1062–1072.

St. Mary, C.M., 1994. Sex allocation in a simultaneous hermaphrodite, the blue-banded goby (*Lythrypnus dalli*): the effects of body size and behavioral gender and the consequences for reproduction. Behav. Ecol. 5, 304–313.

St. Mary, C.M., 1996. Sex allocation in a simultaneous hermaphrodite, the zebra goby *Lythrypnus zebra*: insights gained through a comparison with its sympatric congener, *Lythrypnus dalli*. Environ. Biol. Fishes 45, 177–190.

St. Mary, C.M., 1998. Characteristic gonad structure in the gobiid genus *Lythrypnus* with comparisons to other hermaphroditic gobies. Copeia 1998, 720–724.

Stange, E.A.R., Almeida-Toledo, L.F.D., 1993. Supernumerary B chromosomes restricted to males in *Astyanax scabripinnis* (Pisces, Characidae). Rev. Bras. Genet. 16, 601–615.

Stanley, J.G., 1981. Manipulation of developmental events to produce monosex and sterile fish. The early life history of fish: recent studies. Rapp. P.-V. Reun. Ciem 178, 485–491.

Stanley, J.G., Thomas, A.E., Smitherman, R.O., Shelton, W.L., Grover, J.H., 1978. Absence of sex reversal in unisex grass carp fed methyltestosterone. Symposium on Culture of Exotic Fishes, Aquaculture '78, Atlanta, Georgia (USA).

Stegeman, J.J., 1993. The cytochromes *P*450 in fish. In: Hochachka, P., Mommsen, T. (Eds.), Biochemistry and Molecular Biology of Fishes. Elsevier, pp. 137–158.

Stein, J.D., Phillips, R.B., Devlin, R.H., 2001. Identification of sex chromosomes in chinook salmon (*Oncorhynnchus tshawytscha*). Cytogenet. Cell Genet. 98, 108–110.

Streisinger, G., Walker, C., Dower, N., Knauber, D., Singer, F., 1981. Production of clones of homozygous diploid zebrafish (*Brachydanio rerio*). Nature 291, 293–296.

Strüssmann, C.A., Takashima, F., Toda, K., 1996a. Sex differentiation and hormonal feminization in pejerrey *Odontesthes bonariensis*. Aquaculture 139, 31–45.

Strüssmann, C.A., Moriyama, S., Hanke, E.F., Cota, J.C.C., Takashima, F., 1996b. Evidence of thermolabile sex determination in pejerrey. J. Fish Biol. 48, 643–651.

Strüssmann, C.A., Cota, J.C.C., Phonlor, G., Higuchi, H., Takashima, F., 1996c. Temperature effects on sex differentiation of two South American atherinids, *Odontesthes argentinensis* and *Patagonina hatcheri*. Environ. Biol. Fishes 47, 143–154.

Strüssmann, C.A., Saito, T., Usui, M., Yamada, H., Takashima, F., 1997. Thermal thresholds and critical period of thermolabile sex determination in two atherinid fishes, *Odontesthes bonariensis* and *Patagonina hatcheri*. J. Exp. Zool. 278, 167–177.

Strüssmann, C.A., Saito, T., Takashima, F., 1998. Heat-induced germ cell deficiency in the teleosts *Odontesthes bonariensis* and *Patagonina hatcheri*. Comp. Biochem. Physiol. 119, 637–644.

Subrahmanyam, K., 1969. A karyotypic study of estuarine fish *Boleophthalmus boddaerti* (Pallas) with calcium treatment. Curr. Sci. 38, 437.

Sugama, K., Taniguchi, N., Nabeshima, H., 1989. Frequency of second meiotic division segregation in induced gynogenetic diploid of red sea bream. The Second Asian Fisheries Forum. Proceedings of the Second Asian Fisheries Forum, Tokyo, Japan. The Second Asian Fisheries Forum, Tokyo, Japan, 543–547.

Sumpter, J.P., 1997. Environmental control of fish reproduction: a different perspective. Fish Physiol. Biochem. 17, 25–31.

Sumpter, J.P., Jobling, S., 1995. Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. Environ. Health Perspect. 103, 173–178.

Sundararaj, B.I., Nath, P., 1981. Steroid-induced synthesis of vitellogenin in the catfish, *Heteropneustes fossilis* (Bloch). Gen. Comp. Endocrinol. 43, 201–210.

Sunobe, T., Nakazono, A., 1990. Polygynous mating system of *Trimma okinawae* (Pisces: Gobiidae) at Kagoshima, Japan with a note on sex change. Ethology 84, 133–143.

Sunobe, T., Nakazono, A., 1993. Sex change in both directions by alteration of social dominance in *Trimma okinawae* Pisces Gobiidae. Ethology 94, 339–345.

Suzuki, K., Hioki, S., Tanaka, Y., Iwasa, K., 1979. Spawning behavior, eggs, larvae, and sex reversal of two pomacanthine fish, *Genicanthus lamarck*, and *G. semifasciatus*, in the aquarium. J. Fac. Mar. Sci. Technol., Tokai Univ. 12, 149–165.

Suzuki, R., Oshiro, T., Nakanishi, T., 1985. Survival, growth and fertility of gynogenetic diploids induced in the cyprinid loach, *Misgurnus anguillicaudatus*. Aquaculture 48, 45–55.

Suzuki, A., Taki, Y., Takeda, M., Akatsu, S., 1988a. Multiple sex chromosomes in a monodactylid fish. Jpn. J. Ichthyol. 35, 98–101.
Suzuki, K., Kawauchi, H., Nagahama, Y., 1988b. Isolation and characterization of two distinct gonadotropins from chum salmon pituitary glands. Gen. Comp. Endocrinol. 71, 292–301.
Swain, A., Lovell-Badge, R., 1997. A molecular approach to sex determination in mammals. Acta Paediatr. 423, 46–49.
Swanepoel, A., Meyer, E.H.H., Nel, N.D., 1992. A comparative study of the karyotypes of *Tilapia rendalli*, *Tilapia sparrmanii* and *Oreochromis mossambicus* Cichlidae. S.-Afr. Tydskr. Natuurwet. Tegnol. 11, 105–109.
Swanson, P., Suzuki, K., Kawauchi, H., Dickhoff, W., 1991. Isolation and characterization of two coho salmon gonadotropins, GTH I and GTH II. Biol. Reprod. 44, 28–38.
Szyper, J.P., Anderson, M.J., Richman, N.H., 1991. Preliminary aquaculture evaluation of moi *Polydactylus sexfilis*. Prog. Fish-Cult. 53, 20–25.
Tabata, K., 1991. Induction of gynogenetic diploid males and presumption of sex determination mechanisms in the hirame *Paralichthys olivaceus*. Bull. Jpn. Soc. Sci. Fish. 57, 845–850.
Tabata, K., 1995. Reduction of female proportion in lower growing fish separated from normal and feminized seedlings of hirame *Paralichthys olivaceus*. Fish. Sci. (Tokyo) 61, 199–201.
Takahashi, H., 1975. Masculinization of the gonad of juvenile guppy, *Poecilia reticulata*, induced by 11-keto-testosterone. Bull. Fac. Fish., Hokkaido Univ. 26, 223–234.
Takahashi, H., 1977. Juvenile hermaphroditism in the zebrafish, *Brachydanio rerio*. Bull. Fac. Fish., Hokkaido Univ. 28, 57–65.
Takahashi, H., Shimizu, M., 1983. Juvenile intersexuality in a cyprinid fish, the *Sumatra barb*, *Barbus tetrazona tetrazona*. Bull. Fac. Fish., Hokkaido Univ. 34, 69–78.
Takahashi, M., Tanaka, M., Sakai, N., Adachi, S., Nagahama, Y., 1992. Rainbow trout cholesterol side-chain cleavage cytochrome *P*450 (*P*450scc): cDNA cloning and mRNA expression. Zool. Sci. 9, 1256.
Takamatsu, N., Kanda, H., Ito, M., Yamashita, A., Yamashita, S., Shiba, T., 1997. Rainbow trout SOX9: cDNA cloning, gene structure and expression. Gene 202, 167–170.
Takashima, F., Patino, R., Nomura, M., 1980. Histological studies on the sex differentiation in rainbow trout. Bull. Jpn. Soc. Sci. Fish. 46, 1317–1322.
Takemura, A., Oka, M., 1998. Immunochemical sexing of living yellowfin tuna, *Thunnus albacares* (Bonnaterre), using a vitellogenin-like protein. Aquacult. Res. 29, 245–249.
Takeo, J., Yamashita, S., 1999. Two distinct isoforms of cDNA encoding rainbow trout androgen receptors. J. Biol. Chem. 274, 5674–5680.
Talikina, M.G., 1995. Sex differentiation and gonad development during the first years of life in the bream *Abramis brama* from the Rybinsk water reservoir. Vopr. Ikhtiol. 35, 114–119.
Tanaka, H., 1987. Gonadal sex differentiation in flounder, *Paralichthys olivaceus*. Bull. Natl. Res. Inst. Aquacult. 11, 7–19.
Tanaka, H., 1988. Effects of estradiol-17 beta on gonadal sex differentiation in flounder, *Paralichthys olivaceus*. Bull. Natl. Res. Inst. Aquacult. 13, 17–23.
Tanaka, H., Hirose, K., Nogami, K., Hattori, K., Ishibashi, N., 1990a. Sexual maturation and sex reversal in red spotted grouper, *Epinephelus akaara*. Bull. Natl. Res. Inst. Aquacult. 17, 1–15.
Tanaka, H., Hirose, K., Nogami, K.Y., Hattori, K., Ishibashi, N., 1990b. Sexual maturation and sex reversal in red spotted grouper *Epinephelus akaara*. Bull. Natl. Res. Inst. Aquacult. 17, 1–16.
Tanaka, M., Sakai, N., Nagahama, Y., 1991a. cDNA encoding *P*450 aromatase and *P*450c17 lyase of medaka and rainbow trout; their expression and cloning in the ovary. Program and Abstracts, Second International Marine Biotechnology Conference, Baltimore, USA, p. 93.
Tanaka, M., Telecky, T.M., Fukada, F., Adachi, S., Nagahama, Y., 1991b. Rainbow trout cytochrome *P*450 aromatase: cDNA cloning and expression. Zool. Sci. 8, 1173.
Tanaka, M., Fukada, S., Matsuyama, M., Nagahama, Y., 1995. Structure and promoter analysis of the cytochrome *P*-450 aromatase gene of the teleost fish, medaka (*Oryzias latipes*). J. Biochem. 117, 719–725.
Tanaka, M., Kinoshita, M., Kobayashi, D., Nagahama, Y., 2001. Establishment of medaka (*Oryzias latipes*) transgenic lines with the expression of green fluorescent protein exclusively in germ cells: a useful model to monito germ cells in a live vertebrate. Proc. Natl. Acad. Sci. U. S. A. 98, 2544–2549.

Tan-Fermin, J.D., 1992. Withdrawal of exogenous 17-alpha methyl-testosterone causes reversal of sex-inversed male grouper *Epinephelus suillus* (Bloch and Schneider). Philipp. Sci. 29, 33–39.

Tan-Fermin, J.D., Garcia, L.M.B., Castillo Jr., A.R., 1994. Induction of sex inversion in juvenile grouper, *Epinephelus suillus*, (Valenciennes) by injections of 17 alpha-methyltestosterone. Jpn. J. Ichthyol. 40, 413–420.

Tao, Y.X., Lin, H.R., Van Der Kraak, G., Peter, R.E., 1993. Hormonal induction of precocious sex reversal in the ricefield eel, *Monopterus albus*. Aquaculture 118, 131–140.

Tayamen, M.M., Shelton, W.L., 1978. Inducement of sex reversal in *Sarotherodon niloticus* (Linnaeus). Aquaculture 14, 349–354.

Taylor, C.A., Burr, B.M., 1997. Reproductive biology of the northern starhead topminnow, *Fundulus dispar* (Osteichthyes: Fundulidae), with a review of data for freshwater members of the genus. Am. Midl. Nat. 137, 151–164.

Tchoudakova, A., Callard, G.V., 1998. Identification of multiple CYP19 genes encoding different cytochrome *P*450 aromatase isozymes in brain and ovary. Endocrinology 139, 2179–2189.

Tchoudakova, A., Pathak, S., Callard, G.V., 1999. Molecular cloning of an estrogen receptor beta subtype from the goldfish, *Carassius auratus*. Gen. Comp. Endocrinol. 113, 388–400.

Tesch, F.W., 1977. The Eel. Chapman & Hall, New York, NY.

Thibault, R.E., 1978. Ecological and evolutionary relationships among diploid and triploid unisexual fishes associated with the bisexual species, *Poeciliopsis lucida* (Cyprinodontiformes: Poeciliidae). Evolution 32, 613–623.

Thode, G., 1987. Karyotype analysis of the clingfish, *Lepadogaster candollei* Risso (Gobiesociformes). Cytobios 51, 206–207.

Thode, G., Cano, J., Alvarez, M.C., 1983. A karyological study on four species of *Mediterranean gobiid* fishes. Cytologia 48, 131–138.

Thode, G., Amores, A., Martinez, G., 1994. The karyotype of *Balistes carolinensis* Gmelin (Pisces, Tetrodontiformes) a specialized species. Caryologia 47, 257–263.

Thomas, P., Smith, J., 1993. Binding of xenobiotics to the estrogen receptor of spotted seatrout: a screening assay for potential estrogenic effects. Mar. Environ. Res. 35, 147–151.

Thompson, K.W., Hubbs, C., Edwards, R.J., 1978. Comparative chromosome morphology of the blackbasses. Copeia 1978, 172–175.

Thorgaard, G.H., 1977. Heteromorphic sex chromosomes in male rainbow trout. Science 196, 900–902.

Thorgaard, G.H., 1978. Sex chromosomes in the sockeye salmon: a Y-autosome fusion. Can. J. Genet. Cytol. 20, 349–354.

Thorgaard, G.H., 1983a. Chromosome Set Manipulation and Sex Control in Fish. Academic Press, New York, pp. 405–434.

Thorgaard, G.H., 1983b. Chromosomal differences among rainbow trout populations. Copeia 1983, 650–662.

Thorgaard, G.H., 1992. Application of genetic technologies to rainbow trout. Aquaculture 100, 85–97.

Thorgaard, G.H., Gall, G.A.E., 1979. Adult triploids in a rainbow trout family. Genetics 93, 961–973.

Thorgaard, G., Scheerer, P., Hershberger, W., Myers, J., 1990. Androgenetic rainbow trout produced using sperm from tetraploid males show improved survival. Aquaculture 85, 215–221.

Tiersch, T.R., Simco, B.A., Davis, K.B., Wachtel, S.S., 1992. Molecular genetics of sex determination in channel catfish: studies on SRY, ZFY, Bkm, and human telomeric repeats. Biol. Reprod. 47, 185–192.

Timmermans, L.P.M., Taverne, N., 1989. Segregation of promordial germ cells: their numbers and fate during early development of *Barbus conchonius* (Cyprinidae, Teleostei) as indicated by ^{3}H-Thymidine incorporation. J. Morphol. 202, 225–237.

Tobin, A.J., Sheaves, M.J., Molony, B.W., 1997. Evidence of protandrous hermaphroditism in the tropical sparid *Acanthopagrus berda*. J. Fish Biol. 50, 22–33.

Todo, T., Ikeuchi, T., Kobayashi, T., Nagahama, Y., 1999. Fish androgen receptor: cDNA cloning, steroid activation of transcription in transfected mammalian cells, and tissue mRNA levels. Biochem. Biophys. Res. Commun. 254, 378–383.

Torrans, L., Meriwether, F., Lowell, F., Wyatt, B., Gwinup, P.D., 1988. Sex reversal of *Oreochromis aureus* by immersion in mibolerone, a synthetic steroid. J. World Aquacult. Soc. 19, 97–102.

Trant, J.M., 1995. Isolation and characterization of the cDNA encoding the spiny dogfish shark (*Squalus acanthias*) form of cytochrome *P*450c17. J. Exp. Zool. 272, 25–33.

Trant, J.M., 1996. Functional expression of recombinant spiny dogfish shark (*Squalus acanthias*) cytochrome *P*450c17 (17-alpha-hydroxylase/C-17,20-lyase) in yeast (*Pichia pastoris*). Arch. Biochem. Biophys. 326, 8–14.

Trant, J.M., Thomas, P., 1988. Structure–activity relationships of steroids in inducing germinal vesicle breakdown of Atlantic croaker oocytes in vitro. Gen. Comp. Endocrinol. 71, 307–317.

Trant, J.M., Thomas, P., 1989. Changes in ovarian steroidogenesis in vitro associated with final maturation of Atlantic croaker oocytes. Gen. Comp. Endocrinol. 75, 405–412.

Trant, J.M., Lehrter, J., Gregory, T., Nunez, S., Wunder, J., 1997. Expression of cytochrome *P*450 aromatase in the channel catfish, *Ictalurus punctatus*. J. Steroid Biochem. 61, 393–397.

Trant, J.M., Gavasso, S., Ackers, J., Chung, B.-C., Place, A.R., 2001. Developmental expression of cytochrome *P*450 aromatase genes (CYP19a and CYP19b) in zebrafish fry (*Danio rerio*). J. Exp. Zool. 290, 475–483.

Tremblay, L., Yao, X., Van Der Kraak, G., 1995. Interactions of the environmental estrogens nonylphenol and beta-sitosterol with liver estrogen receptors in fish. Proceedings of the Fifth International Symposium On The Reproductive Physiology of Fish. Fish Symposium 95, Austin, TX, USA, p. 202.

Trombka, D., Avtalion, R., 1993. Sex determination in tilapia—a review. Isr. J. Aquacult. 45, 26–37.

Trudeau, V.L., 1997. Neuroendocrine regulation of gonadotrophin II release and gonadal growth in the goldfish, *Carassius auratus*. Rev. Reprod. 2, 55–68.

Trudeau, V.L., Sloley, B.D., Peter, R.E., 1993a. Testosterone enhances gaba and taurine but not *N*-methyl-DL-aspartate stimulation of gonadotropin secretion in the goldfish possible sex steroid feedback mechanisms. J. Neuroendocrinol. 5, 129–136.

Trudeau, V.L., Murthy, C.K., Habibi, H.R., Sloley, B.D., Peter, R.E., 1993b. Effects of sex steroid treatments on gonadotropin-releasing hormone-stimulated gonadotropin secretion from the goldfish pituitary. Biol. Reprod. 48, 300–307.

Truscott, B., 1983. Steroid metabolism in fish: II. Testosterone metabolites in the bile of the marine winter flounder *Pseudopleuronectes americanus* and the freshwater Atlantic *Salmon Salmo* salar. Gen. Comp. Endocrinol. 51, 460–470.

Tsangridis, A., Filippousis, N., 1992. Growth pattern of picarel *Spicara smaris* L. Centracanthidae a protogynous species. Cybium 16, 232–243.

Tuan, P.A., Mair, G.C., Little, D.C., Beardmore, J.A., 1999a. Sex determination and the feasibility of genetically male tilapia production in the Thai-Chitralada strain of *Oreochromis niloticus* L. Aquaculture 173, 257–269.

Tuan, P.A., Mair, G.C., Little, D.C., Beardmore, J.A., 1999b. Sex determination and the feasibility of genetically male tilapia production in the Thai-Chitralada strain of *Oreochromis niloticus* (L.). Aquaculture 173, 257–269.

Turner, B.J., Diffoot, N., Rasch, E.M., 1992. The callichthyid catfish *Corydoras aeneus* is an unresolved diploid–tetraploid sibling species complex. Ichthyol. Explor. Freshwaters (Munchen) 3, 17–23.

Tyler, C.R., Van der Eerden, B., Jobling, S., Panter, G., Sumpter, J.P., 1996. Measurement of vitellogenin, a biomarker for exposure to oestrogenic chemicals, in a wide variety of cyprinid fish. J. Comp. Physiol., B 166, 418–426.

Tyler, C.R., Pottinger, T.G., Coward, K., Prat, F., Beresford, N., Maddix, S., 1997. Salmonid follicle-stimulating hormone (GtH I) mediates vitellogenic development of oocytes in the rainbow trout, *Oncorhynchus mykiss*. Biol. Reprod. 57, 1238–1244.

Ueda, T., Ojima, Y., 1984a. Sex chromosomes in the rainbow trout *Salmo gairdneri*. Bull. Jpn. Soc. Sci. Fish. 50, 1499–1504.

Ueda, T., Ojima, Y., 1984b. Sex chromosomes in the kokanee salmon, *Oncorhynchus nerka*. Bull. Jpn. Soc. Sci. Fish 50, 1495–1498.

Ueda, H., Young, G., Crim, L.W., Kambegawa, A., Nagahama, Y., 1983. 17 alpha,20 beta-Dihydroxy-4-pregnen-3-one: plasma levels during sexual maturation and in vitro production by the testes of amago salmon (*Oncorhynchus rhodurus*) and rainbow trout (*Salmo gairdneri*). Gen. Comp. Endocrinol. 51, 106–112.

Ueno, K., Kang, J.-H., 1992. Multiple sex chromosomes in the redfin velvetfish, *Hypodytes rubripinnis*. Jpn. J. Ichthyol. 39, 170–173.

Ueno, K., Ota, K., Kobayashi, T., 2001. Heteromorphic sex chromosomes of lizardfish (Synodontidae): focus on the ZZ–W1W2 system in *Trachinocephalus myops*. Genetica 111, 133–142.

Uribe-Alcocer, M., Montes-Perez, R., Diaz-Jaimes, P., 1994. Chromosome complement of *Eleotris pisonis* (Gobiidae; Perciformes) from Mexico: a new case of heteromorphic sex chromosomes in fish. Cytobios 77, 183–187.

Uwa, H., Ojima, Y., 1981. Detailed and banding karyotype analyses of the medaka, *Oryzias latipes*, in cultured cells. Proc. Jpn. Acad., Ser. B 57, 39–43.

Uyeno, T., 1973. A comparative study of chromosomes in the teleostean fish order Osteoglossiformes. Jpn. J. Ichthyol. 20, 211–217.

Uyeno, T., Miller, R.R., 1971. Multiple sex chromosomes in a Mexican cyprinodontid fish. Nature 231, 452–453.

Val, A.L., de Almeida-Val, V.M.F., 1995. Fishes of the Amazon and Their Environment: Physiological and Biochemical Aspect. Springer-Verlag, Berlin, Germany, 245 pp.

Valcarcel, A., Brunner, P., Maggese, M.C., 1993. B-chromosome polymorphism in the South American catfish, *Rhamdia sapo*. Aquaculture 110, 111–118.

Van Den Hurk, R., Slof, G.A., 1981. A morphological and experimental study of gonadal sex differentiation in the rainbow trout, *Salmo gairdneri*. Cell. Tissue Res. 218, 487–497.

Van der Kraak, G., Lin, H.R., Donaldson, E.M., Dye, H.M., Hunter, G.A., 1983. Effects of LH-RH and des-Gly super(10)(D-Ala super(6))LH-RH-ethylamide on plasma gonadotropin levels and oocyte maturation in adult female coho salmon (*Oncorhynchus kisutch*). Gen. Comp. Endocrinol. 49, 470–476.

Van der Kraak, G., Dye, H.M., Donaldson, E.M., 1984. Effects of LH-RH and Des-Gly10[D-Ala6]LH-RH-ethylamide on plasma sex steroid profiles in adult female coho salmon (*Oncorhynchus kisutch*). Gen. Comp. Endocrinol. 55, 36–45.

Van der Kraak, G., Dye, H.M., Donaldson, E.M., Hunter, G.A., 1985. Plasma gonadotropin, 17 beta-estradiol, and 17 alpha,20 beta-dihydroxy-4-pregnen-3-one levels during luteinizing hormone-releasing hormone analogue and gonadotropin induced ovulation in coho salmon (*Oncorhynchus kisutch*). Can. J. Zool. 63, 824–833.

Van der Kraak, G., Sorensen, P.W., Stacey, N.E., Dulka, J.G., 1989. Periovulatory female goldfish release three potential pheromones: 17 alpha,20 beta-dihydroxyprogesterone, 17 alpha,20 beta-dihydroxyprogesterone glucuronide, and 17 alpha-hydroxyprogesterone. Gen. Comp. Endocrinol. 73, 452–457.

Van der Kraak, G., Rosenblum, P.M., Peter, R.E., 1990. Growth hormone-dependent potentiation of gonadotropin-stimulated steroid production by ovarian follicles of the goldfish. Gen. Comp. Endocrinol. 79, 233–239.

Van der Kraak, G., Suzuki, K., Peter, R.E., Itoh, H., Kawauchi, H., 1992a. Properties of common carp gonadotropin I and gonadotropin II. Gen. Comp. Endocrinol. 85, 217–229.

Van Der Kraak, G.J., Munkittrick, K.R., McMaster, M.E., Portt, C.B., Chang, J.P., 1992b. Exposure to bleached kraft pulp mill effluent disrupts the pituitary–gonadal axis of white sucker at multiple sites. Toxicol. Appl. Pharmacol. 115, 224–233.

Van Doorn, W.A., 1962. Geschlachtsbeinvloeding door temperature. Het Aquarium 32, 208–209.

Van Eenennaam, A.L., Murray, J.D., Medrano, J.F., 1998a. Synaptonemal complex analysis in spermatocytes of white sturgeon, *Acipenser transmontanus* Richardson (Pisces, Acipenseridae), a fish with a very high chromosome number. Genome 41, 51–61.

Van Eenennaam, A.L., Murray, J.D., Medrano, J.F., 1998b. Mitotic analysis of the North American white sturgeon, *Acipenser transmontanus* Richardson (Pisces, Acipenseridae), a fish with a very high chromosome number. Evolution 52, 266–271.

Van Eenennaam, A.L., Murray, J.D., Medrano, J.F., 1999a. Karyotype of the American green sturgeon. Trans. Am. Fish. Soc. 128, 175–177.

Van Eenennaam, A.L., Van Eenennaam, J.P., Medrano, J.F., Doroshov, S.I., 1999b. Evidence of female heterogametic genetic sex determination in white sturgeon. J. Hered. 90, 231–233.

Van Vuren, J.H.J., Soley, J.T., 1990. Some ultrastructural observations of Leydig and Sertoli cells in the testis of *Tilapia rendalli* following induced testicular recrudescence. J. Morphol. 206, 57–64.

Van Winkoop, A., Timmermans, L.P.M., 1992. Phenotypic changes in germ cells during gonadal development of the common carp *Cyprinus carpio* an immunohistochemical study with anti-carp spermatogonia monoclonal antibodies. Histochemistry 98, 289–298.

Van Winkoop, A., Timmermans, L.P.M., Goos, H.J.T., 1994. Stimulation of gonadal and germ cell development

in larval and juvenile carp (*Cyprinus carpio* L.) by homologous pituitary extract. Fish Physiol. Biochem. 13, 161–171.

Varadaraj, K., 1990. Production of monosex male *Oreochromis mossambicus* (Peters) by administering 19-norethisterone acetate. Aquacult. Fish. Manage. 21, 133–135.

Varadaraj, K., Pandian, T.J., 1989. First report on production of supermale tilapia by integrating endocrine sex reversal with gynogenetic technique. Curr. Sci. (Bangalore) 58, 434–441.

Varadaraj, K., Kumari, S.S., Pandian, T.J., 1994. Comparison of conditions for hormonal sex reversal of Mozambique tilapias. Prog. Fish-Cult. 56, 81–90.

Vasil'ev, V.P., Vasil'eva, E.D., 1992. Karyological evidence for the specific identities of *Neogobius kessleri* (Guenther) and *Neogobius gorlap* Iljin (Pisces, Gobiidae). Dokl. An. 324, 898–900.

Vasil'ev, V.P., Vasil'eva, E.D., Osinov, A.G., 1990. Reticular speciation in vertebrates diploid–triploid–tetraploid complex in the genus Cobitis (Cobitidae): 3. Origin of the triploid form. J. Ichthyol. 30, 543–550.

Vasil'eva, E.D., Osinov, A.G., Vasil'ev, V.P., 1989. The problem of reticulate speciation in vertebrates: the diploid–triploid–tetraploid complex in the genus *Cobitis* (Cobitidae). J. Ichthyol. 29, 35–47.

Vasil'yev, V.I., Vasil'yeva, E.D., Osinov, A.G., 1991. The problem of reticulate species formation in vertebrates of the diploid–triploid–tetraploid complex in the genus *Cobitis* (Cotibidae): 4. Tetraploid form. J. Ichthyol. 31, 21–35.

Vasil'yeva, Y., 1990. On morphological divergence of the gynogenetic and bisexual forms of the goldfish, *Carassius auratus* (Cyprinidae, Pisces). J. Ichthyol. 30, 22–37.

Vera Cruz, E.M., Mair, G.C., 1994. Conditions for effective androgen sex reversal in *Oreochromis niloticus* (L.). Aquaculture 122, 2–3.

Vidalis, K., Tsimenidis, N., 1997. Some aspects of reproduction and sexuality in the spotcheek emperor, *Lethrinus rubrioperculatus*, in waters off the Ryukyu Islands Age determination and growth of picarel (*Spicara smaris*) from the Cretan continental shelf (Greece). Ichthyol. Res. 44, 201–212.

Vilia, P., Canario, A.V.M., 1995. Effect of LHRH on sex reversal and steroid levels on gilthead seabream (*Sparus aurata*). Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish, Univ. Austin, TX (USA). Fish Symposium 95, Austin, TX, USA, p. 329.

Vissotto, P.C., Foresti, F., Oliveira, C., 1997. A ZZ/ZW sex chromosome system in *Imparfinis mirini* (Pisces, Siluriformes). Cytologia 62, 61–66.

Vitturi, R., Catalano, E., 1988. Karyotypes in two species of the genus *Hippocampus* (Pisces: Syngnatiformes). Mar. Biol. 99, 119–121.

Vitturi, R., Rasotto, M.B., 1990. Karyotype analysis of *Cottus gobio* L. Pisces Cottidae. Cytobios 62, 81–86.

Vitturi, R., Mazzola, A., Macaluso, M., Catalano, E., 1986. Chromosomal polymorphism associated with Robertsonian fusion in *Seriola dumerili* (Risso, 1810) (Pisces: Carangidae). J. Fish Biol. 29, 529–534.

Vitturi, R., E, C., A, M., T, C., 1988. Karyological studies in *Coris julis* (Pisces, Labridae). Genetica 76, 219–223.

Vitturi, R., E, C., F, L., 1991a. Evidence of heteromorphic sex chromosomes in *Zeus faber* (Pisces, Zeiformes): nucleolus organizer regions and C-banding pattern. Cytobios 272, 37–43.

Vitturi, R., Catalano, E., Lafargue, F., 1991b. Evidence for heteromorphic sex chromosomes in *Zeus faber* Pisces Zeiformes nucleolus organizer regions and C-banding pattern. Cytobios 68, 37–44.

Vitturi, R., Catalano, E., Schillaci, A., 1993a. Karyotypic characterization of 16 *Microchirus ocellatus* L. specimens Pisces Soleidae using conventional and silver staining nors. Caryologia 46, 41–45.

Vitturi, R., Catalano, E., Colombera, D., 1993b. Chromosome analysis of *Bothus podas* Pisces Pleuronectiformes from the Mediterranean Sea. J. Fish Biol. 43, 221–227.

Vizziano, D., Le Gac, F., Fostier, A., 1995. Synthesis and regulation of 17 alpha-hydroxy-20 beta-dihydroprogesterone in immature males of *Oncorhynchus mykiss*. Fish Physiol. Biochem. 14, 289–299.

Vizziano, D., Le Gac, F., Fostier, A., 1996a. Effect of 17 beta-estradiol, testosterone, and 11-ketotestosterone on 17,20 beta-dihydroxy-4-pregnen-3-one production in the rainbow trout testis. Gen. Comp. Endocrinol. 104, 179–188.

Vizziano, D., Fostier, A., Le Gac, F., Loir, M., 1996b. 20 beta-hydroxysteroid dehydrogenase activity in non-flagellated germ cells of rainbow trout testis. Biol. Reprod. 54, 1–7.

Vogt, R.C., Bull, J.J., 1982. Genetic sex determination in the spiny softshell *Trionyx spiniferus* (Testudines: Trionychidae). Copeia, 699–700.

Vrijenhhoek, R.C., 1993. The origin and evolution of clones versus the maintenance of sex in *Poeciliopsis*. J. Hered. 84, 388–395.
Vriz, S., Lovell-Badge, R., 1995. The zebrafish Zf-Sox 19 protein: a novel member of the Sox family which reveals highly conserved motifs outside of the DNA-binding domain. Gene 153, 275–276.
Wachtel, S., Demas, S., Tiersch, T., Pechan, P., Shapiro, D., 1991. Bkm satellite DNA and ZFY in the coral reef fish *Anthias squamipinnis*. Genome 34, 612–617.
Wada, H., Shimada, A., Fukamachi, S., Naruse, K., Shima, A., 1998. Sex-linked inheritance of the If locus in the medaka fish (*Oryzias latipes*). Zool. Sci. 15, 123–126.
Waltz, C.W., Roumillat, W.A., Wenner, C.A., 1982. Biology of the whitebone porgy, *Calamus leucosteus*, in the South Atlantic Bight. Fish. Bull.-NOAA 80, 863–874.
Wang, L.H., Tsai, C.L., 2000. Effects of temperature on the deformity and sex differentiation of tilapia, *Oreochromis mossambicus*. J. Exp. Zool. 286, 534–537.
Wang, J., Zhao, X., 1994. Chromosome studies of *Synechogobius ommathurus* (Osteichthyes: Gobiidae). Mar. Sci. 4, 47–50.
Waring, C.P., Moore, A., 1997. Sublethal effects of a carbamate pesticide on pheromonal mediated endocrine function in mature male Atlantic salmon (*Salmo salar* L.) parr. Fish Physiol. Biochem. 17, 1–6.
Warner, R.R., 1982. Mating systems, sex change and sexual demography in the rainbow wrasse, *Thalassoma lucasanum*. Copeia, 653–661.
Warner, R.R., 1988. Sex change in fishes: hypotheses, evidence, and objections. Environ. Biol. Fishes 22, 81–90.
Warner, R., Hoffman, S., 1980. Local population size as a determinant of mating system and sexual composition in two tropical marine fishes (*Thalassoma* spp.). Evolution 34, 508–518.
Warner, R.R., Lejeune, P., 1985. Sex change limited by paternal care: a test using four Mediterranean labrid fishes, genus *Symphodus*. Mar. Biol. 87, 89–99.
Warner, R., Robertson, D., 1978. Sexual patterns in the labroid fishes of the western Caribbean: 1. The wrasses (Labridae). Smithson. Contrib. Zool. 255, 1–26.
Warner, R.R., Swearer, S.E., 1991. Social control of sex change in the bluehead wrasse, *Thalassoma bifasciatum* (Pisces: Labridae). Biol. Bull. 181, 199–204.
Wassef, E.A., 1991. Comparative growth studies on *Lethrinus lentjan* Lacepede 1802 and *Lethrinus mahsena* Forsskal 1775 Pisces Lethrinidae in the Red Sea. Fish. Res. 11, 75–92.
Watanabe, K.I., Suzuki, N., 1996. Sex differentiation, sexual maturity and the spawning season of the red tilefish *Branchiostegus japonicus* on the Pacific Coast of Tokushima Prefectures. Bull. Jpn. Soc. Sci. Fish./Nippon Suisan Gakkaishi 62, 406–413.
Watanabe, M., Tanaka, M., Kobayashi, D., Yoshiro, Y., Oba, Y., Nagahama, Y., 1999. Medaka (*Oryzias latipes*) FTZ-F1 potentially binds to promoter regions of *P*-450 aromatase: cDNA cloning and functional characterization. Mol. Cell. Endocrinol. 149, 221–228.
Webb, C.J., 1986. Karyology of the Indo-Pacific *Parioglossus raoi* (Herre) (Teleostei: Gobioidei) from Fiji. Aust. J. Mar. Freshwater Res. 37, 347–351.
Webb, R.O., Kingsford, M.J., 1992. Protogynous hermaphroditism in the half-banded sea perch, *Hypoplectrodes maccullochi* (Serranidae). J. Fish Biol. 40, 951–961.
Wei, G., Mahowald, A.P., 1994. The germline: familiar and newly uncovered properties. Annu. Rev. Genet. 28, 309–324.
Weis, S., Schartl, M., 1998. The macromelanophore locus and the melanophore oncogene xmrk are separate genetic entities in the genome of *Xiphophorus*. Genetics 149, 1909–1920.
Weller, P.A., Critcher, R., Goodfellow, P.N., German, J., Ellis, N.A., 1995. The human Y chromosome homologue of XG: transcription of a naturally truncated gene. Hum. Mol. Genet. 4, 859–868.
Wernerus, F.M., Tessari, V., 1991. The influence of population density on the mating system of *Thalassoma pavo* a protogynous Mediterranean labrid fish. Mar. Ecol. 12, 361–368.
Wester, P.W., Canton, J.H., 1986. Histopathological study of *Oryzias latipes* (medaka) after long-term beta-hexachlorocyclohexane exposure. Aquat. Toxicol. 9, 21–45.
Western, P.S., Harry, J.L., Graves, J.A., Sinclair, A.H., 1999. Temperature-dependent sex determination: upregulation of SOX9 expression after commitment to male development. Dev. Dyn. 214, 171–177.
Williams, J.G.K., Reiter, R.S., Young, R.M., Scolnik, P.A., 1993. Genetic mapping of mutations using phenotypic pools and mapped RAPD markers. Nucleic Acids Res. 11, 2697–2702.

Wing, O., 1922. One-sided masculine and sex-linked inheritance in *Lebistes reticulates*. J. Genet. 12, 137–144.
Withler, R.E., McPhail, J.D., Devlin, R.H., 1986. Electrophoretic polymorphism and sexual dimorphism in the freshwater and anadromous threespine sticklebacks (*Gasterosteus aculeatus*) of the Little Campbell River, British Columbia. Biochem. Genet. 24, 701–713.
Wohlfarth, G.W., Wedekind, H., 1991. The heredity of sex determination in tilapias. Aquaculture 92, 2–3.
Woiwode, J., 1977. Sex reversal of *Tilapia zillii* by ingestion of methyltestosterone. Tech. Pap. Ser. Bur. Fish. Aquat. Resour. 1, 1–5.
Wolf, L.E., 1931. The history of the germ cells in the viviparous teleost *Platypoecilius maculates*. J. Morphol. 52, 115–153.
Wolf, U., 1998. The serologically detected H–Y antigen revisited. Cytogenet. Cell Genet. 80, 232–235.
Wright, J.M., 1993. DNA fingerprinting of fishes. In: Hochachka, P., Mommsen, T. (Eds.), Biochemistry and Molecular Biology of Fishes. Elsevier, Amsterdam, New York, pp. 57–91.
Wu, C.J., Ye, Y.Z., Chen, R.D., 1986. Genome manipulation in carp (*Cyprinus carpio* L.). Aquaculture 54, 57–61.
Wu, W., Li, C., Liu, G., Xu, D., Liu, C., Xie, J., Shan, C., 1988. Studies on tetraploid hybrid between red common carp (*Cyprinus carpio*) and grass carp (*Ctenopharyngodon idellus*) and its backcross triploid. Acta Hydrobiol. Sin. 12, 355–363.
Wu, C.J., Chen, R.D., Ye, Y.Z., Huang, W.Y., 1990. Production of all-female carp and its application in fish cultivation. Genet. Aquacult. III 85, 1–4.
Wu, C., Ye, Y., Chen, R., Liu, X., 1993. An artificial multiple triploid carp and its biological characteristics. Genetics in Aquaculture IV. Aquaculture 111, 255–262.
Xia, D.Q., Liu, A.Z., Wu, T.T., Sun, Y., 1990. Study of clones of commercial fish. Aquaculture 85, 327–328.
Xia, Z., Patino, R., Gale, W.L., Maule, A.G., Densmore, L.D., 1999. Cloning, in vitro expression, and novel phylogenetic classification of a channel catfish estrogen receptor. Gen. Comp. Endocrinol. 113, 360–368.
Xiao, Y., Liu, Y., 1995. Study on the histology in sex changing from intersex to male of *Monopterus albus* (Zuiew). J. Fish. China 19, 297–304.
Yamamoto, T., 1953. Artificially induced sex reversal in genotypic males of the medaka (*Oryzias latipes*). J. Exp. Zool. 123, 571–594.
Yamamoto, T., 1955. Progeny of artificially induced sex reversals of male genotype (XY) in the medaka (*Oryzias latipes*) with special reference to YY-male. Genetics 40, 406–419.
Yamamoto, T., 1958. Artificial induction of functional sex-reversal in genotypic females of the medaka (*Oryzias latipes*). J. Exp. Zool. 137, 227–262.
Yamamoto, T., 1964. The problem of viability of YY zygotes in the medaka, *Oryzias latipes*. Genetics 50, 45–58.
Yamamoto, T., 1969. Sex differentiation. In: Hoar, W., Randall, D. (Eds.), Fish Physiology. Academic Press, pp. 117–175.
Yamamoto, E., 1999. Studies on sex-manipulation and production of cloned populations in hirame, *Paralichthys olivaceus* (Temminck et Schlegel). Aquaculture 173, 235–246.
Yamamoto, T., Kajishima, T., 1969. Sex-hormone induction of reversal of sex differentiation in the goldfish and evidence for its male heterogamety. J. Exp. Zool. 168, 215–222.
Yamashita, M., Kajiura, H., Tanaka, T., Onoe, S., Nagahama, Y., 1995. Molecular mechanisms of the activation of maturation promoting factor during oocyte maturation. Dev. Biol. 168, 62–75.
Yamashita, A., Suzuki, S., Fujitani, K., Kojima, M., Kanda, H., Ito, M., Takamatsu, N., Yamashita, S., Shiba, T., 1998. cDNA cloning of a novel rainbow trout SRY-type HMG box protein, rtSox23, and its functional analysis. Gene 209, 1–2.
Yamazaki, F., 1976. Application of hormones in fish culture. J. Fish. Res. Board Can. 33, 948–958.
Yamazaki, F., 1983a. Chromosomes and abnormal embryogenesis in salmonids. Can. Trans. Fish. Aquat. Sci. 4958, 1–15.
Yamazaki, F., 1983b. Sex control and manipulation in fish. Aquaculture 33, 329–354.
Yan, H.Y., 1984. Occurrence of spermatozoa and eggs in the gonad of a tidewater silverside, *Menidia beryllina*. Copeia, 544–545.
Yang, Z.a., Gui, J., Zhu, L., Liang, S., Jiang, Y., 1994. Cytological studies on two differential development modes of the allogynogenetic silver crucian carp multiple tetraploid. Acta Zool. Sin. 40, 69–74.

Yano, K., 1995. Reproductive biology of the black dogfish, *Centroscyllium fabricii*, collected from waters off western Greenland. J. Mar. Biol. Assoc. U. K. 75, 285–310.

Yaron, Z., 1995. Endocrine control of gametogenesis and spawning induction in the carp. Aquaculture 129, 49–73.

Ye, Y., Wu, Q., Chen, R., 1989. Studies on cytology of crosses between grass carp and (common) carp: asynchronization between nucleus and cytoplasm in distant hybridization of fishes. Acta Hydrobiol. Sin. 13, 234–239.

Yeoh, C.G., Schreck, C.B., Feist, G.W., Fitzpatrick, M.S., 1996. Endogenous steroid metabolism is indicated by fluctuations of endogenous steroid and steroid glucuronide levels in early development of the steelhead trout (*Oncorhynchus mykiss*). Gen. Comp. Endocrinol. 103, 107–114.

Yeung, W.S., Chan, S.T., 1985a. The in vitro metabolism of radioactive progesterone and testosterone by the gonads of the protandrous *Rhabdosargus sarba* at various sexual phases [published erratum appears in 1987 Apr;66(1):160]. Gen. Comp. Endocrinol. 59, 171–183.

Yeung, W.S.B., Chan, S.T.H., 1985b. The in vitro metabolism of radioactive progesterone and testosterone by the gonads of the protandrous *Rhabdosargus sarba* at various sexual phases. Gen. Comp. Endocrinol. 59, 171–183.

Yeung, W.S., Chan, S.T., 1987a. A radioimmunoassay study of the plasma levels of sex steroids in the protandrous, sex-reversing fish *Rhabdosargus sarba* (Sparidae). Gen. Comp. Endocrinol. 66, 353–363.

Yeung, W.S., Chan, S.T., 1987b. The plasma sex steroid profiles in the freshwater, sex-reversing teleost fish, *Monopterus albus* (Zuiew). Gen. Comp. Endocrinol. 65, 233–242.

Yeung, W.S.B., Chan, S.T.H., 1987c. A radioimmunoassay study of the plasma levels of sex steroids in the protandrous, sex-reversing fish *Rhabdosargus sarba* (Sparidae). Gen. Comp. Endocrinol. 66, 353–363.

Yeung, W.S.B., Chan, S.T.H., 1987d. The gonadal anatomy and sexual pattern of the protandrous sex-reversing fish, *Rhabdosargus sarba* (Teleostei: Sparidae). J. Zool. 212, 521–532.

Yeung, W.S.B., Chen, H., Chan, S.T.H., 1993a. In-vivo effects of Olh and Lhrh-analog on sex reversal and plasma sex steroid profiles in the female *Monopterus albus*. Gen. Comp. Endocrinol. 90, 23–30.

Yeung, W.S.B., Chen, H., Chan, S.T.H., 1993b. The in-vitro metabolism of radioactive androstenedione and testosterone by the gonads of the protogynous *Monopterus albus* at different sexual phases a time-course and seasonal study. Gen. Comp. Endocrinol. 89, 313–322.

Yeung, W.S.B., Chen, H., Chan, S.T.H., 1993c. Effects of Lh and Lhrh-analog on gonadal development and in-vitro steroidogenesis in the protogynous *Monopterus albus*. Gen. Comp. Endocrinol. 89, 323–332.

Yogo, Y., 1985. Studies on the sexual maturation and reproductive ecology in three protogynous fishes. Rep. Fish. Res. Lab., Kyushu Univ. 7, 37–83.

Yoon, C., Kawakami, K., Hopkins, N., 1997. Zebrafish *vasa* homologue RNA is localized to the cleavage planes of 2- and 4-cell-stage embryos and is expressed in the primordial germ cells. Development 124, 3157–3166.

Yoshikawa, H., Oguri, M., 1978. Sex differentiation in a cichlid, *Tilapia zillii*. Bull. Jpn. Soc. Sci. Fish. 44, 313–318.

Yoshikuni, M., Nagahama, Y., 1994. Involvement of an inhibitory G-protein in the signal transduction pathway of maturation-inducing hormone (17 alpha, 20 beta-dihydroxy-4pregnen-3-one) action in rainbow trout (*Oncoorhynchus mykiss*) oocytes. Dev. Biol. 166, 615–622.

Yoshizaki, G., Takeuchi, Y., Sakatani, S., Takeuchi, T., 2000. Germ cell-specific expression of green fluorescent protein in transgenic rainbow trout under control of the rainbow trout vasa-like gene promoter. Int. J. Dev. Biol. 44, 323–326.

Young, P.C., Martin, R.B., 1985. Sex ratios and hermaphroditism in nemipterid fish from northern Australia. J. Fish Biol. 26, 273–287.

Young, G., Kagawa, H., Nagahama, Y., 1982. Oocyte maturation in the amago salmon (*Oncorhynchus rhodurus*): in vitro effects of salmon gonadotropin, steroids, and cyanoketone (an inhibitor of 3 beta-hydroxy-Delta super(5)-steroid dehydrogenase). J. Exp. Zool. 224, 265–275.

Young, G., Kagawa, H., Nagahama, Y., 1983a. Evidence for a decrease in aromatase activity in the ovarian granulosa cells of amago salmon (*Oncorhynchus rhodurus*) associated with final oocyte maturation. Biol. Reprod. 29, 310–315.

Young, G., Ueda, H., Nagahama, Y., 1983b. Estradiol-17 beta and 17 alpha,20 beta-dihydroxy-4-pregnen-3-one

production by isolated ovarian follicles of amago salmon (*Oncorhynchus rhodurus*) in response to mammalian pituitary and placental hormones and salmon gonadotropin. Gen. Comp. Endocrinol. 52, 329–335.

Young, G., Crim, L.W., Kagawa, H., Kambegawa, A., Nagahama, Y., 1983c. Plasma 17 alpha,20 beta-dihydroxy-4-pregnen-3-one levels during sexual maturation of amago salmon (*Oncorhynchus rhodurus*): correlation with plasma gonadotropin and in vitro production by ovarian follicles. Gen. Comp. Endocrinol. 51, 96–105.

Young, W.P., Wheeler, P.A., Fields, R.D., Thorgaard, G.H., 1996. DNA fingerprinting confirms isogenicity of androgenetically derived rainbow trout lines. J. Hered. 87, 77–80.

Young, W.P., Wheeler, P.A., Coryell, V.H., Keim, P., Thorgaard, G.H., 1998. A detailed linkage map of rainbow trout produced using doubled haploids. Genetics 148, 839–850.

Zacharewski, T.R., Berhane, K., Gillesby, B.E., Burnison, B.K., 1995. Detection of estrogen- and dioxin-like activity in pulp and paper mill black liquor and effluent using in vitro recombinant receptor/reporter gene assays. Environ. Sci. Technol. 29, 2140–2146.

Zechel, C., U, S., A, A., F, A., 1988. v-erbB related sequences in *Xiphophorus* that map to melanoma determining Mendelian loci and overexpress in a melanoma cell line. Oncogene 3, 605–617.

Zelenkov, V.M., 1982. Earl gametogenesis and sex differentiation in the perch, *Perca fluviatilis*. J. Ichthyol. 21, 124–130.

Zelinskiy, Y.M.I.M., 1985. Analysis of chromosomal variability and polymorphism in Atlantic salmon, *Salmo salar*, of Lake Onega. J. Ichthyol. 25, 70–77.

Zhang, Q., Arai, K., 1996. Flow cytometry for DNA contents of somatic cells and spermatozoa in the progeny of natural tetraploid loach. Fish. Sci. 62, 870–877.

Zhang, F., Oshiro, T., Takashima, F., 1992. Chromosome synapsis and recombination during meiotic division in gynogenetic triploid ginbuna, *Carassius auratus* langsdorfii. Jpn. J. Ichthyol. 39, 151–156.

Zhang, Q., Nakayama, I., Fujiwara, A., Kobayashi, T., Oohara, I., Masaoka, T., Kitamura, S., Devlin, R.H., 2001. Sex identification by male-specific growth hormone pseudogene (GH-Y) in *Oncorhynchus masou* complex and a related hybrid. Genetica 111, 111–118.

Zhao, W., Wright, R.S., 1986. Sex steroids production by vitellogenic ovarian follicles of Atlantic salmon (*Salmo salar*) in vitro. J. Fish. China 10, 389–394.

Zhao, W., Wright, R.S., 1988. A study on sex steroids of male Atlantic salmon *Salmo salar* in vivo and in vitro. Oceanol. Limnol. Sin./Haiyang Yu Huzhao 19, 532–538.

Zhao, W., Tan, Y., Jiang, R., Kang, C., 1988. Changes of sex steroids during induced ovulation of silver carp (*Hypophthalmichthys molitrix*). Acta Hydrobiol. Sin. 12, 212–218.

Zhou, J., Shen, J., Liu, M., 1983. A cytological study on the gynogenesis of Fang-Zheng Crucian carp of Heilongjiang Province. Acta Zool. Sin. 29, 11–15.

Zhou, R., Zhang, Q., Tiersch, T.R., Cooper, R.K., 2001. Four members of the Sox gene family in channel catfish. J. Fish Biol. 58, 891–894.

Zhu, Y., 1987. A study of the development of the gonads of *Tilapia nilotica*. J. Fujian Teach. Univ., Nat. Sci. Ed./ Fujian Shifan Daxue Xuebao 3, 74–81.

Zohar, Y., Abraham, M., Gordin, H., 1978. The gonadal cycle of the captivity-reared hermaphroditic teleost *Sparus aurata* (L.) during the first two years of life. Ann. Biol. Anim., Biochim., Biophys. 18, 877–882.

Zou, J.J., Trudeau, V.L., Cui, Z., Brechin, J., Mackenzie, K., Zhu, Z., Houlihan, D.F., Peter, R.E., 1997. Estradiol stimulates growth hormone production in female goldfish. Gen. Comp. Endocrinol. 106, 102–112.